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The Relationships among 24-Hour Urinary Cortisol, Energy Intake, Body Composition, and Training on the Menstrual Cycles of Elite Female Synchronized Swimmers

by

Jennifer Susan Ringrose

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Faculty of Physical Education and Recreation

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **The Relationships among 24-Hour Urinary Cortisol, Energy Intake, Body Composition, and Training on the Menstrual Cycles of Elite Female Synchronized Swimmers** in partial fulfillment of the requirements for the degree of Master of Science.

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May 26, 1998
Date

Abstract

Although there are many benefits to exercise, there are also some potential health detriments. Menstrual disorders have been frequently observed in athletic females. Several predisposing factors have been associated with menstrual disorders, yet the relationships between these variables are unclear. Some of these factors were examined in this study. Specifically, relationships among urinary cortisol, psychological stress, coping skills, energy intake, body composition, menstrual cycle characteristics, and early follicular phase luteinizing hormone (LH) were investigated over a 10 month period in national calibre female synchronized swimmers (SS; n=9) and sedentary controls (CG; n=8). Age and gynecologic age were not different between groups (age(yrs)[mean±SE]: SS=17.2±0.5, C=17.9±0.6; gynecologic age(yrs): SS=3.4±0.6, CG=5.0±0.7). VO_2 max (ml/kg/min) was higher ($p<.05$) in SS vs. CG, averaging 45.11±1.4 and 35.34±2.0, respectively. Urinary free cortisol (UFC) excretion, Derogatis Stress Profile (DSP) scores, Ways of Coping Questionnaire (WOC) scores, energy intake, the sum of 5 skinfold thicknesses (SOS), body mass index (BMI), menstrual cycle characteristics (interval length, period length, and blood loss), and serum LH were determined at several time points. BMI and SOS were initially lower ($p<.05$) in SS versus CG (BMI=20.9±0.6, 23.6±1.2; SOS (mm)=66.4±5.0, 94.1±5.5, respectively). SOS decreased to 55.2±2.4 ($p<.05$) in the SS during the study and remained unchanged in the CG. No significant differences were demonstrated in any of the other variables between groups or testing times.

Case studies for SS were examined to explore relationships at the individual level. The case studies showed important inter-individual data that were not apparent in the

group means. Urinary free cortisol for subject 101 increased as this athlete approached the National competition. The interval between subsequent menstrual periods increased throughout the study and LH pulse amplitude, area, and mean level decreased. This profile is most similar to the hypothesized adaptations to a year of synchronized swimming training. Subject 103 recorded her lowest body weight and body fat results concurrently with her lowest caloric intake and her longest menstrual cycle length. This pattern may indicate that this subject was not consuming enough energy to maintain her menstrual function at the time of the last testing session prior to the National Championships. Subject 108 had primary amenorrhea until the months preceding the study and was therefore likely still pubescent during the study. Her dietary intake of calories and macronutrients was low. This low energy intake coupled with this subject's immature reproductive axis may explain her menstrual irregularities. Subject 109's results were similar to the SS group mean on most measures. Her relatively advanced gynecologic age, mature coping skills and experience in this sport and the training it involves may have protected this athlete from the variability in menstrual cycle patterns seen in the other case study athletes.

While the group data show few significant differences, some of the case studies show cause for concern. One potential explanation for the lack of group differences may be the young age of the participants in this study. LH and UFC have not been previously studied in this age range. Any effects of training in this study may have been masked by the effects of maturation.

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List of Abbreviations

ACTH-Adrenalcorticotrophic Hormone
AN-Anorexia Nervosa
ANOVA-Analysis of Variance
BMC-Bone Mineral Content
BMI-Body Mass Index
CG-Control Group
CHD-Coronary Heart Disease
CRH- Corticotrophic Releasing Hormone
CT-Computed Tomography
CSTF-Canadian Standardized Test of Fitness
CV- Coefficient of Variation
DEXA-Dual-Energy X-ray Absorptiometry
DSP-Derogatis Stress Profile
E_2-Estradiol
EFP-Early Follicular Phase
EOP-Endogenous Opiate Center
FFM-Fat Free Mass
FM-Fat Mass
FSH-Follicle Stimulating Hormone
GABA-Gamma-Amino-Butyric Acid
GnRH-Gonadotropin Releasing Hormone
HA-Hypothalamic Amenorrhea
HDL-High Density Lipoproteins
HPA-Hypothalamic-Pituitary-Adrenal Axis
HPO-Hypothalamic-Pituitary-Ovarian Axis
HR-Heart Rate
IV-Intravenous
LDL-Low Density Lipoproteins
LH-Luteinizing Hormone
MLP-Mid Luteal Phase
MRI-Magnetic Resonance Imaging
P-Progesterone
PBAC-Pictorial Blood loss Assessment Charts
QC-Quality Control
RIA-Radioimmunoassay
SOS-Sum of 5 Skinfold Thicknesses
SS-Synchronized Swimmers
UFC-Urinary Free Cortisol
VO_2 max-Maximum Oxygen Consumption
WOC-Ways of Coping Questionnaire

Chapter One
Introduction

Overview

In the past 3 decades, the participation of women in sports has increased dramatically (Thein & Thein, 1996; Van De Loo & Johnson, 1995). This increased participation in sports has generally led to improved health, physical fitness, and well-being for participants (Nattiv, Agostini, Drinkwater, & Yeager, 1994; Thein & Thein). However, some women have become vulnerable to one or more medical disorders that have come to be described in the literature as the *female athlete triad* (Nattiv et al.). The female athlete triad refers to the combination of disordered eating, menstrual disorders, and decreased bone mineral density. This triad has the potential to have a deleterious effect on many female athletes.

Young women are faced with societal pressure to be thin. In addition to societal pressures, some female athletes face pressure to be lean for performance or for aesthetic reasons (Marshall & Harber, 1996; Sundgot-Borgen, 1994). This has led to a high prevalence of disordered eating tendencies among athletes (Nattiv et al., 1994). Disordered eating tendencies can include behaviours such as binge eating, laxative use, vomiting, diuretic use, secretive eating and fasting. Unfortunately, these tendencies have the potential to progress into eating disorders such as anorexia nervosa and bulimia (Thein & Thein, 1996; Sundgot-Borgen, 1993). Disordered eating tendencies may lead to menstrual dysfunction (Nattiv et al.; Thein & Thein).

The prevalence of menstrual disorders in athletic women varies, however it is clear that young women training intensively have a much greater risk of developing

menstrual disorders than their sedentary peers (Erdelyi, 1976; Glass, Deuster, Kyle, Yahiro, Vigersky, & Shoomaker, 1987; Highet, 1989; Loucks, Vaitukaitis, Cameron, Rogol, Skrinar, & Limacher, 1992; Ronkainen, Pakainen, & Kauppila, 1984). The incidence of amenorrhea in the general population is 2-5%, whereas in athletic populations, the range is from 3.4-66% (Nattiv et al., 1994). The incidence of other menstrual disorders is difficult to quantify since these disorders are asymptomatic in that a woman may have a menstrual bleed yet have an anovulatory cycle or a short or insufficient luteal phase cycle (Bullen, Skrinar, Beitins, von Mering, Turnbull, & McArthur, 1985; Prior & Vigna, 1991).

The primary locus of altered menstrual function is likely the gonadotropin releasing hormone (GnRH) secreting neurons of the arcuate nucleus (Veldhuis, 1990). There are many predisposing factors which have been linked to the suppression of the GnRH pulse generator. Among these factors are body composition (low body fat, low body weight) (Bale, 1994; Frisch, 1987; Sinning & Little, 1987; Warren, 1992), exercise type, elevated exercise volume and high exercise intensity (Bullen et al., 1985; Cumming & Rebar, 1983; Kaiserauer, Snyder, Sleeper, & Zierath,1989; Ronkainen, Pakarinen, & Kauppila, 1984, Ronkainen, Pakarinen, Kirkinen, & Kauppila, 1985), a history of irregular menstruation (Lindholm, Hagenfeldt, & Ringertz, 1994; Loucks, 1990; Warren, 1992), hypercortisolism (De Souza, Luciano, Arce, Demers, & Loucks, 1994; Loucks, Mortola, Girton, & Yen, 1989), physical stress (environmental, hemorrhage, pharmacological, surgical, exercise) (Cameron, Helmreich, & Schreihofer, 1993; Chrousos, 1992; De Souza. Maguire, Maresh, Kraemer, Rubin, & Loucks, 1991;

Heinrichs, Menzaghi, Pich, Britton, & Koob, 1995; Pacak, Palkovits, Kvetnansky, Yadid, Kopin, & Goldstein, 1995; Wade, Schneider, & Li, 1996), psychological stress (Stratakis, Gold, & Chrousos, 1995), increased endogenous opioid secretion (Genazzani & Petraglia, 1989; Gindoff & Ferin, 1987; Samuels, Sanborn, Hofeldt, & Robbins, 1991; Veldhuis, 1990), increased levels of catechol estrogens (Russell, Mitchell, Musey, & Collins, 1984b), changes in neurotransmitter secretion (Judd, Wong, Saloniklis, Maiden, Yeap, Filmer, & Michailov, 1995; Quigley, Sheehan, Casper, & Yen, 1980, Veldhuis, 1990), and inadequate nutrition (Cumming, Wheeler, & Harber, 1994; Rosetta, 1993; Wade et al.).

These predisposing factors can be divided into 3 categories: 1) energetic challenges (inadequate nutrition, body fatness, and intense exercise training), 2) stress (psychological and physical), and 3) other (opioid secretion, neurotransmitter secretion, catechol estrogen secretion, menstrual history, undetermined factors) (Figure 1.1). These factors will be discussed in detail in chapter two. The underlying mechanism by which these predisposing factors lead to menstrual disorders has not been identified, however it is likely a multifactorial mechanism which compensates for energy and stress challenges by suppressing non-essential processes like reproduction, growth, and fat storage (Wade et al., 1996).

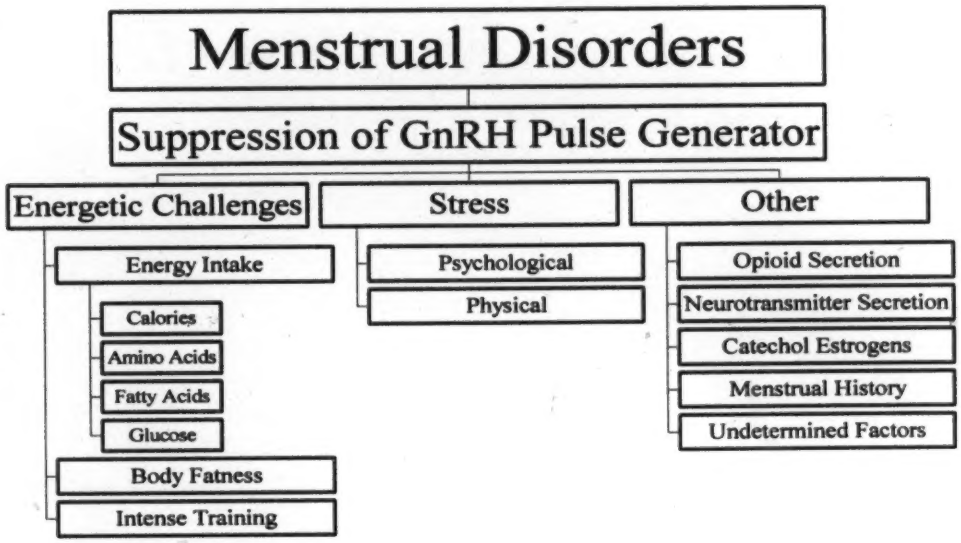


Figure 1.1 Overview of the potential predisposing factors which may lead to menstrual disorders

Menstrual disorders are a female health concern. Initially, when oligomenorrhea and amenorrhea were first observed in female athletes, infertility was the only major health concern, a transient product of training (Drinkwater, 1992). Then came reports of secondary changes: premature bone loss, stress fractures, and negatively altered blood lipid levels, which augmented the health risks associated with menstrual disorders. Decreased bone mineral density is the third component of the female athlete triad.

Bone mineral content is typically higher in athletes who perform weight-bearing exercise than in non-athletes (Carbon, 1992). Weight training may be a better stimulus for increasing bone mineral content than running or swimming (Heinrich, Going, Pamenter, Perry, Boyden, & Lohman, 1990). However, athletes with amenorrhea or other menstrual disorders often have lower bone mineral density than their eumenorrheic

peers (Carbon; Drinkwater, Nilson, Chesnut, Bremner, Shainholtz, & Southworth, 1984). Skeletal sites predominated by trabecular rather than cortical bone are most affected by hormonal insufficiency (Carbon). Menstrual disorders in adolescence may be particularly detrimental since the majority of adult bone mass is deposited during these years (Blimkie, Rice, Webber, Levy, & Parker, 1992). Lost bone mineral density may be partially recovered, however likely not to the level of individuals who have not had menstrual disorders (Iketani, Kiriike, Nakanishi, & Nakasuji, 1995). Hormone replacement therapy may be initiated in women with menstrual disorders to prevent bone loss (Marshall, 1994). However, proper doses of replacement hormones have not been established for young women as they have been in post-menopausal women (Marshall). Furthermore, studies on the long term effects of menstrual disorders on bone mineral content have not been conducted.

The focus of research on the female athlete triad should be to reveal the predisposing factors and causal mechanisms which lead to these disorders. Then, future training programs can be designed for female athletes which strive to promote health while attempting to avoid menstrual disorders, decreased bone mineral density, and disordered eating.

Statement of the Problem

Previous research has produced the following observations which provided the basis for this study:

1) Athletic women are generally leaner and weigh less than their sedentary counterparts.
2) Insufficient nutrition is related to reproductive dysfunction.

3) Menstrual disorders are more prevalent in athletic women than in the general population.
4) Some health consequences of menstrual disorders may be irreversible.
5) Athletic women generally have a more active hypothalamic-pituitary-adrenal axis (HPA) than their sedentary counterparts, and hyperactivation of this axis has been shown to suppress the hypothalamic-pituitary-ovarian axis (HPO).

The relationships among these previous research findings have not been elucidated. Previous research has not examined these issues concurrently and prospectively. The goal of the present study, therefore, was to investigate these issues concurrently and prospectively in an attempt to determine how they are related.

Study Objectives

The primary purpose of this study was to conduct a prospective (10 month) investigation of the relationships among 24-hour urinary cortisol, energy intake, body composition, and training on the menstrual cycles of elite female synchronized swimmers. To investigate these relationships, the following parameters were studied for 10 months in 9 elite female synchronized swimmers and 8 sedentary females (see Figure 1.2 for an overview of the study):

1) 24-hour urinary cortisol levels: Urine was collected for a 24-hour period 3 times during the study and analyzed for urinary free cortisol (UFC). UFC was compared between groups, within groups, and between testing times.

2) Psychological stress: 2 questionnaires were administered 4 times during the study. Subscales for types of stress and coping styles were compared between groups, within groups, and between testing times.

3) Energy intake of calories, protein, carbohydrate, and fat: 3-day energy intake records were completed 4 times during the study and analyzed for mean 3-day calories, grams of protein, carbohydrate, and fat, and the percentage of the total energy intake that was comprised of protein, carbohydrate, and fat. Mean values were compared between groups, within groups, and between testing times.

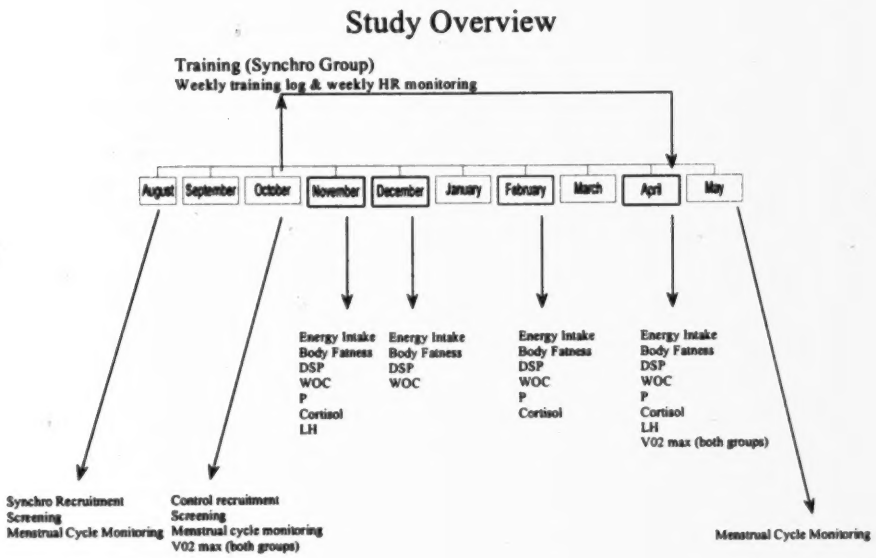
4) Body weight, body mass index, height, and sum of skinfolds: Height, weight, and 5 skinfold thicknesses were determined 4 times during the study. These variables were compared between groups, within groups, and between testing times.

5) Training volume and intensity of elite synchronized swimmers: This parameter was measured in the synchronized swimmers only. Training volumes were determined from both athlete and coach training logs, while training intensity was determined by recording training heart rates for each athlete 5-6 times during the study. Weekly training hours were recorded for each week of the study.

6) Menstrual cycle length, the number of days each menstrual period lasts, and the quantity of blood lost during each menstrual period: Menstrual cycle length and menstrual period length were determined from menstrual logs. The quantity of menstrual blood lost was estimated using pictorial blood loss charts completed during each menstrual period for the duration of the study (Higham, O'Brien, & Shaw, 1990). These parameters were compared within groups between months and between groups.

7) Luteal phase salivary progesterone: Saliva samples were collected daily from day 12 of the menstrual cycle to the subsequent day 1 during 3 menstrual cycles. Saliva was analyzed for progesterone content. Area under the progesterone curve was compared between groups, within groups, and between testing times.

8) Early follicular phase serum luteinizing hormone pulsatility: Blood samples were collected every 10 minutes for an 8 hour period during the early follicular phase of 2 menstrual cycles during the study. The blood was analyzed for serum luteinizing hormone (LH). LH pulse frequency, amplitude, area, and the interval between pulses were compared between groups, within groups and between testing times.



Key: VO_2 max = maximum oxygen consumption, DSP = Derogatis Stress Profile, WOC = Ways of Coping Questionnaire, P = progesterone, LH = luteinizing hormone

Figure 1.2 Overview of the study

Variables

Independent Variables

1. Body Composition
2. Stress
3. Training
4. Energy Intake

Dependent Variables

1. LH pulsatility
2. Luteal Phase Progesterone
3. Menstrual Cycle Frequency and Duration
4. Quantity of Menstrual Flow

Statistical Hypothesis

Ho: No relationships exist among the independent and dependent variables during 10 months of synchronized swimming training ($\mu_1=\mu_2$).

Ha: Relationships do exist among the independent and dependent variables during 10 months of synchronized swimming training ($\mu_1\neq\mu_2$).

μ_1 = Baseline Data

μ_2 = Other Testing Data

The level of significance was set *a priori* at $\alpha=0.05_{2\text{ tail}}$.

Justification

Choice of Topic

The endocrine changes that lead to menstrual disorders are not clearly understood. Many predisposing factors have been related to these disorders, yet no absolute conclusions have been made. The health consequences of menstrual disorders include: 1) decreased bone mineral density, 2) infertility, and potentially, 3) negative changes in blood lipid profiles.

Many women enjoy their athletic careers between the ages of 15-30 years old. These are also the years of maximal skeletal mass formation (Highet, 1989; Van De Loo & Johnson, 1995). Athletes with menstrual disorders may be hypoestrogenic and/or have low progesterone levels. Low levels of both hormones have been associated with accelerated bone density loss (Highet; Prior, 1990). Therefore, athletic women with depressed reproductive hormone levels may not have the opportunity to achieve maximal bone density. Accelerated bone density loss may also make the female athlete more vulnerable to bone fractures. The reversibility of this bone loss is questionable (Carbon, 1992; Highet; Nattiv et al., 1994).

Infertility is another consequence of menstrual disorders (Constantini, 1994). Exercise associated infertility is usually reversible (Constantini; Prior & Vigna, 1985).

Low levels of estradiol, found in athletes with menstrual disorders, may also adversely affect blood lipid profiles. Exercise is associated with elevated high-density lipoproteins and decreased low-density lipoproteins. Estradiol also enhances lipolysis in muscle and adipose tissue, and inhibits gluconeogenesis and glycogenolysis

(Constantini). Conversely, insufficient estradiol concentrations may negatively affect plasma lipids and potentially accelerate the development of atherosclerosis (Constantini).

Given these health consequences of menstrual disorders, and the increasing numbers of female participants in competitive sport, it is essential to study the characteristics of various groups of female athletes in an effort to reveal the cause of menstrual disorders and the preventive steps which can be taken to eradicate this problem from the future generations of female athletes.

Choice of Sport

Synchronized swimming is a unique sport which combines the emphasis on leanness prevalent in judged, aesthetic sports with the requirement of body fatness for buoyancy. Little research has been conducted on synchronized swimmers. Menstrual disorders have been documented in swimmers (Russell, Mitchell, Musey, & Collins, 1984a; Russell et al., 1984b; Sanborn, Martin, & Wagner, 1982) with an incidence of 12.3% (Sanborn et al.). Although synchronized swimmers cross-train by swimming, synchronized swimming is a unique sport and should be investigated independently. Only one study has examined menstrual function in synchronized swimmers. Ouellette, MacVicar, and Harlan (1986) conducted a 10 month longitudinal study recording percent body fat, height, weight, training hours, and menstrual cycle length in 9 synchronized swimmers, 6 gymnasts, 7 track athletes, 1 marathon runner, 1 volleyball player, and 40 control subjects. No significant differences in menstrual cycle length were found between the athletes and the control group (Ouellette et al.). Menstrual cycle length in isolation is not an accurate marker of menstrual cycle function. To accurately assess

menstrual cycle function, hormonal measures are required. Therefore, the prevalence of menstrual disorders in elite synchronized swimmers has not been accurately assessed.

Choice of Study Design

The longitudinal nature of this study was advantageous in that it tracked athletes belonging to one club through a typical training season. This design encompassed many of the variables which may affect the elite female synchronized swimmer's menstrual cycle. Since the synchronized swimmers were selected from the same club, inter-individual training variations were minimized. There were some disadvantages to this design. Emotional stress from home and school could not be controlled. Also, due to the invasive nature and long duration of this study, attrition in both groups was a risk. Attrition was limited to one control subject by keeping all subjects fully informed about each aspect of the study and by making every effort to be available for, and to answer any questions that the subjects may have had.

The results obtained from this study will be generalizable to elite senior and 15-17 year old National Age Group synchronized swimmers. The teams examined and their training are likely representative of elite synchronized swimming training programs across Canada. The information gained from this study, therefore, will be valuable to future synchronized swimming coaches, athletes and administrators who may use the findings to design future training programs.

Definitions

For the purposes of this study, the following definitions will apply:

Chronically trained synchronized swimmers: athletes who have trained for a minimum of 3 years at a competitive provincial or national "A" level, 10 months of the year, for a minimum of 15 hours per week.

Feedback loops: control of stimulatory hormone secretion by target organ secretion of hormones which "feedback" to the stimulatory organ to either provide further stimulation (positive feedback), or to slow or stop further stimulation (negative feedback). Three types of feedback loops have been described: ultra-short loop (hypothalamus to hypothalamus/ovary to ovary); short loop (pituitary to hypothalamus); and long loop (peripheral organ to pituitary or hypothalamus) (Ferin, Jewelewicz, &Warren, 1993).

Luteal phase abnormalities: include luteal phases which do not last for at least 9 days between the surge of luteinizing hormone and the next menstrual period (shortened luteal phase) and luteal phases which do not display the characteristic parabolic curve of progesterone levels (insufficient luteal phase) (Bullen et al., 1985).

Menstrual disorders: include primary amenorrhea, delayed menarche, luteal phase abnormalities, oligomenorrhea, and secondary amenorrhea.

Oligomenorrhea: menstrual cycles that occur at intervals from 39-90 days (Loucks & Horvath, 1985).

Primary amenorrhea: the absence of menarche beyond age 16 years (Loucks & Horvath, 1985).

Regular/Eumenorrhea: menstrual cycles that occur consistently at intervals of 25-38 days (Loucks & Horvath, 1985).

Secondary amenorrhea: the absence of menstruation in women who have previously had at least one episode of menstrual bleeding for a length of time equivalent to a total of at least 3 of her previous cycle lengths (Marshall, 1994); menstrual cycles that occur at intervals greater than 90 days (Loucks & Horvath, 1985).

Sedentary individuals: will refer to individuals who participate in physical activity less than 3 times per week for a duration of 30 minutes or less and who have a baseline VO_2max of less than 39 mL/kg/min. This VO_2max value is considered to be average for Canadian females 15-19 years old performing a predicted VO_2max test (CSTF Operations Manual, 1986).

Stress: a state of disharmony or threatened homeostasis where counteracting/reestablishing forces, or adaptational responses consisting of an extraordinary repertoire of physical or mental reactions that attempt to counteract the effects of stressors are applied in order to reestablish homeostasis (Chrousos & Gold, 1992). This repertoire of reactions includes the activation of the HPA (Heinrichs et al., 1995).

Assumptions

To conduct this study it is assumed that the athletes selected for study constituted a representative sample of elite female synchronized swimmers. It must also be assumed that the training season to be studied (1996-1997 season) was representative of a typical

training season. Participant response was assumed to be truthful and it was assumed that the subjects followed the instructions given to them with regards to the study.

Limitations

This study was limited to one geographical location. Thus, the small sample size, and having only one athletic group, may limit the generalizability of this study. A final limitation is the possibility that there are other, yet undertermined factors, which are major influences on menstrual function that have not been assessed in this study and that could explain the results of this investigation differently.

Chapter Two
Literature Review
Part I-The Normal Menstrual Cycle

The information contained in this section, unless otherwise indicated, is a summary of The Menstrual Cycle (1993) written by Ferin, Jewelewicz, and Warren.

Overview

The human menstrual cycle is the product of many highly regulated hormonal events coordinated by precise feedback loops. The purpose of this cycle is to produce an oocyte which may be fertilized for the purpose of reproduction. This cycle has 3 phases: 1) the follicular phase when the follicle grows, 2) the ovulatory phase when the mature oocyte is released into the reproductive tract, and 3) the luteal phase (figure 2.1) when the corpus luteum secretes hormones to prepare the endometrium for implantation of the fertilized egg. If implantation does not occur, the corpus luteum wanes which leads to menstruation and the start of a new cycle. Each cycle usually lasts between 25-38 days. By convention, the first day of menstruation is counted as day one. The follicular phase is variable in length but usually lasts approximately 14 days. The luteal phase is more consistent in length and usually lasts 12-15 days.

Major Hormones of the Menstrual Cycle

There are 4 major hormones which characterize the human menstrual cycle: 2 anterior pituitary hormones, luteinizing hormone (LH), and follicle-stimulating hormone (FSH); and 2 ovarian hormones, estradiol (E_2), and progesterone (P).

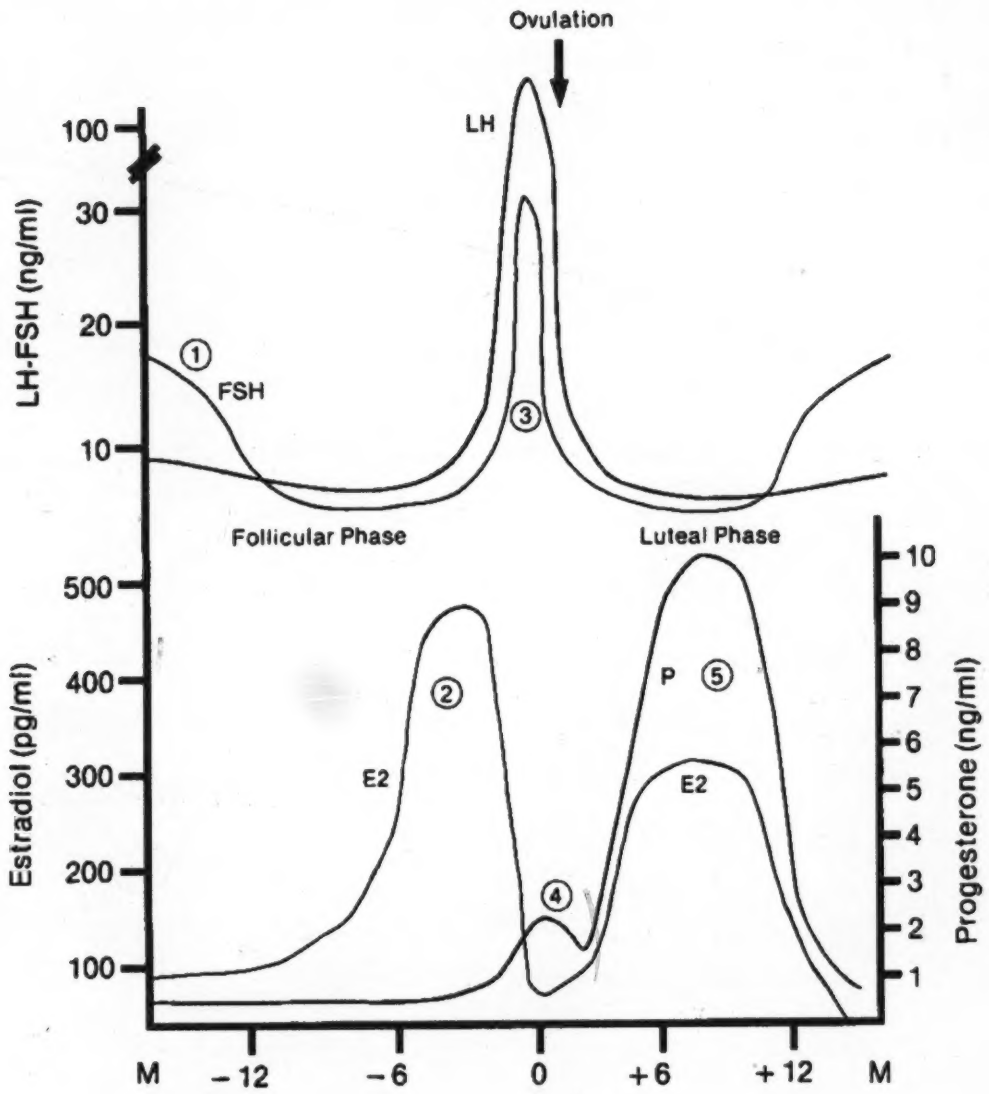


Figure 2.1 **The menstrual cycle: 1. the early follicular phase, 2. the late follicular phase, 3. the preovulatory gonadotropin surge, 4. the preovulatory progesterone rise, and 5. the luteal phase estradiol and progesterone secretory curve. From The Menstrual Cycle (p.6) by Ferin, Jewelewicz, and Warren (1993).**

The anterior pituitary hormones LH and FSH are gonadotropins. The most significant contribution of LH to the menstrual cycle is the preovulatory LH surge which occurs at the end of the follicular phase. This surge lasts for approximately 48 hours. Ovulation occurs approximately 18 hours after the LH peak or 36 hours after the beginning of the LH surge. Follicle-stimulating hormone also rises at the end of the follicular phase, however the rise of this hormone is much smaller than the LH surge. In the day(s) preceding or on the day of menstruation, there is a significant rise in FSH. In the early follicular phase, FSH reaches its peak concentration approximately 24 hours after menstrual flow starts. This is the only time in the menstrual cycle when FSH concentrations exceed LH concentrations.

Estradiol secretion is low during the early follicular phase and increases approximately 1 week prior to the mid cycle LH surge. This increase is gradual and then increases rapidly. Estradiol peaks at the onset of the LH surge. Within hours of the LH surge, E_2 concentrations decrease. Estradiol concentrations rise again when the corpus luteum appears. Progesterone is insignificant in the follicular phase until the preovulatory progesterone rise which occurs approximately 12 hours prior to the LH surge. Progesterone concentration then reaches a plateau for approximately 12 hours and then falls. Progesterone levels rise again approximately 36 hours after the onset of the LH surge. During the luteal phase (LP), both P and E_2 increase to reach a maximum concentration 6-9 days after the mid-cycle LH surge.

The Neuroendocrine Component

Gonadotropin-releasing hormone (GnRH) is released from neurons in the arcuate nucleus region of the medial basal hypothalamus into the pituitary portal circulation (Knobil, 1980). Gonadotropin-releasing hormone is released approximately once every 60-90 minutes and has a 2-4 minute half life (Crowley, Filicori, Spratt, & Santoro, 1985; Knobil). Because of this short half life, GnRH concentrations in the peripheral circulation are very dilute and as a result this hormone cannot be measured directly, without the insertion of a catheter into the median eminence of the hypothalamus. Animal studies are conducted in this manner (Knobil), however for ethical reasons, human studies requiring the measurement of endogenous GnRH are impossible. Gonadotropin-releasing hormone is essential for gonadotropin release. Therefore, LH and FSH pulses, which are presumably preceded by GnRH release, are used as markers for GnRH (Crowley et al.).

Gonadotropin-releasing hormone acts on receptors on gonadotrophs to catalyze gonadotropin release. Gonadotropin response to GnRH is rapid. Gonadotropin-releasing hormone affects the LH pulse rate but not the duration (Genazzani, Rodbard, Forti, Petraglia, Baraghini, & Genazzani, 1990). Luteinizing hormone release varies in sensitivity to GnRH depending on the steroidal environment around the pituitary gland. Maximal sensitivity to GnRH occurs at mid cycle. The change in pituitary sensitivity to GnRH during the follicular phase is a result of increased E_2 concentrations. At its peak concentration, E_2 requires approximately 12 hours to exert the mid cycle enhancement of LH responsiveness to GnRH. There is a significant increase in LH pulse frequency from

the early follicular phase to the late follicular phase (Backstrom, McNeilly, Leask, & Baird, 1982). Pituitary sensitivity to GnRH during the mid luteal phase is equal to that seen in the late follicular phase. Luteinizing hormone sensitivity to GnRH is therefore increased in the presence of E_2 or P. Estradiol and P affect both the amplitude and the frequency of LH pulses (Genazzani et al.). Follicle stimulating hormone pulsatility is more difficult to quantify than LH since FSH has a longer half life than LH in the peripheral circulation, and because smaller amounts of FSH are released in response to GnRH. There is an inverse relationship between E_2 and FSH. Estradiol suppresses FSH at the pituitary level. Therefore, FSH is more evident when E_2 is low. The frequency of the GnRH pulse may affect the genetic transcription of gonadotropin hormones. Slow pulses may result in FSH secretion whereas fast pulses may result in LH secretion from the anterior pituitary.

The Ovarian Component

There are 2 functional cyclical units within the ovary: 1) the follicle; which provides necessary support for the oocyte; and 2) the corpus luteum, which prepares the endometrium for implantation of an embryo. Both ovarian structures secrete steroid hormones. There are 3 types of ovarian steroids: 1) the progestins, 2) the androgens (androstenedione and testosterone), and 3) the estrogens (estrone and estradiol). These steroids are produced by 3 cell types in the ovaries in response to gonadotropin stimulation.

During the follicular phase of the menstrual cycle, many ovarian follicles grow. These follicles consist of 3 cell types: 1) granulosa cells, 2) theca cells, and 3) luteal

cells. Granulosa cells form the inner envelope around the oocyte and are mainly FSH responsive. These cells metabolize androgens to estrogens during the mid-late follicular phase. During the late follicular phase, these cells synthesize P in response to FSH and LH stimulation. Theca cells surround the granulosa cell layer and appear only when the follicle grows. These cells synthesize androgens, and P in response to LH stimulation. The preovulatory P rise is a result of the P secreted from the theca cells. The luteal cells secrete both E_2 and P in response to LH stimulation.

There are 2 possible fates for ovarian follicles, atresia or ovulation. Most growing follicles do not ovulate, they undergo atresia. A follicle may also degenerate even before it is recruited. Ovulation occurs when the walls of the dominant grown follicle rupture. The ovulation process is initiated by the LH surge which is the result of the positive feedback of E_2 on LH secretion. After ovulation, the ruptured follicle is transformed into the corpus luteum. The corpus luteum is mature 5 days after ovulation. Approximately 7-9 days after ovulation, if the oocyte is not fertilized, regression of the corpus luteum begins. This regression is characterized by decreased steroid secretion, decreased vascularization, and a decreased number of secretory granules. By 12-15 days after ovulation, the corpus luteum atrophies and a new cycle begins.

Hypothalamic-Pituitary-Ovarian Communication

Follicular Phase

A normal menstrual cycle requires the synchronization between the hypothalamus, the anterior pituitary and the ovary (figure 2.2). Gonadotropin-releasing hormone causes LH and FSH secretion which in turn cause morphological then secretory

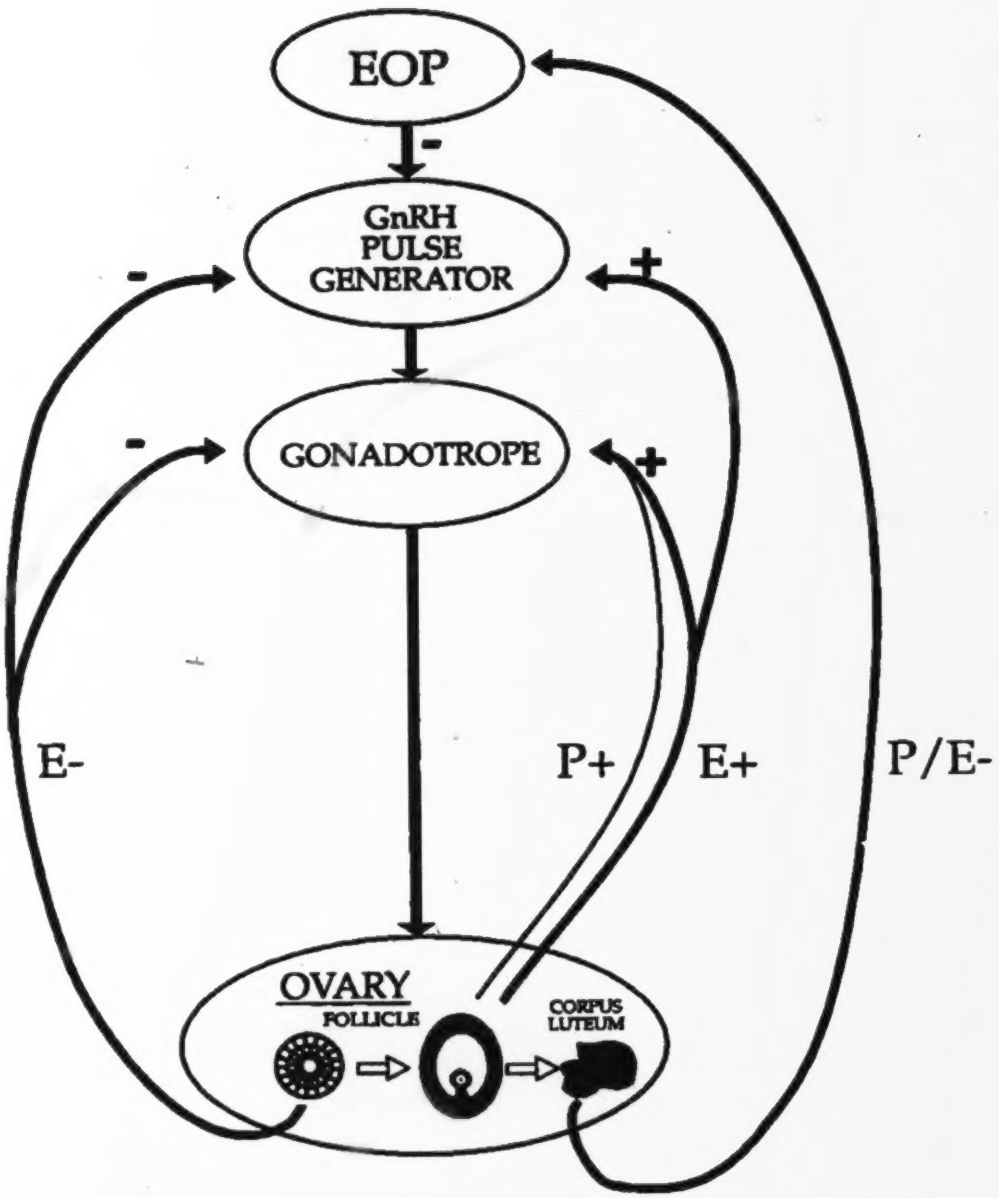


Figure 2.2 **The HPO and the sites of ovarian steroid feedback. EOP represents the endogenous opiate center. From The Menstrual Cycle (p.59) by Ferin, Jewelewicz, and Warren (1993)**

changes in the ovary. Follicle-stimulating hormone initiates this process by recruiting the dominant ovarian follicle from 3-5 antral follicles. On day 1 of the menstrual cycle, there is no distinguishable dominant follicle. By day 3, a dominant follicle can be distinguished by size and/or increased granulosa cell mitotic activity. Follicle-stimulating hormone stimulates E_2 production in granulosa cells. Estradiol suppresses FSH below concentrations necessary to nurture other follicles. On day 6, the dominant follicle is distinguishable from other follicles in development and vascularization. The increased vascularization ensures that LH is delivered preferentially to the dominant follicle. Luteinizing hormone stimulates the production of androgens in the theca cells which is then converted to E_2 in the granulosa cells. Therefore, by the late follicular phase, over 90% of circulating E_2 is due to 1 dominant follicle. There is a negative feedback loop of E_2 on gonadotropin secretion during the early follicular phase. This feedback loop may only affect LH pulse amplitude, not pulse frequency. During the late follicular phase, E_2 stimulates LH and FSH secretion. For E_2 to exert this positive feedback, 2 conditions must be met: the E_2 concentration must be greater than 300-500 pg/mL, and E_2 must be present in these concentrations for more than 48 hours.

Luteinizing hormone and FSH pulsatility in the early follicular phase lay the foundation for the remainder of the menstrual cycle. Schweiger, Laessle, Tuschl, Brooks, Krusche, and Pirke (1989) found that decreased LH pulsatility and to a lesser extent FSH pulsatility in the early follicular phase were associated with decreased E_2 and P secretion in the luteal phase of the menstrual cycle in normally menstruating women. Cumming, Vickovik, Wall, and Fluker (1985) showed that LH pulse amplitude, frequency and area

under the curve in the early follicular phase, were all diminished in eumenorrheic runners. Luteinizing hormone, therefore, is a sound marker of reproductive performance. Luteinizing hormone has a shorter half life in plasma than FSH, making FSH pulses more difficult to detect (Backstrom et al., 1982). Furthermore, the concentration of FSH in the blood is much less than the concentration of LH during the early follicular phase (Backstrom et al.). In the early follicular phase (days 1-4), LH pulse frequency approximates the pulse frequency observed in the ovariectomized conditions, indicating that the ovarian steroids do not affect LH pulse frequency at this point in the cycle. Early follicular phase LH pulsatility is an accurate marker of the integrity of the follicular phase.

Gonadotropin Surge

The LH surge causes a decrease in the proliferation of granulosa cells. In addition, there is a decrease in E_2 due to decreased androgen production in the theca cells. During the LH surge, follicular fluid contains decreased concentrations of E_2 and androgens, and the follicle begins to secrete P. As the preovulatory increase in P occurs, E_2 remains low.

Luteal Phase

After ovulation, the corpus luteum is formed. Adequate follicular formation is essential for normal corpus luteum function. The main function of the corpus luteum is to secrete E_2 and P. Progesterone blocks the long loop positive feedback between E_2 and LH that was present in the late follicular phase. This blockade of the positive feedback loop can only occur if the concentration of P exceeds the concentration of E_2. Estradiol

and P pulses are simultaneous in the luteal phase of the menstrual cycle which suggests co-secretion by the corpus luteum. Pathophysiology in the luteal phase of the cycle may range from decreased progesterone secretion to shortened luteal phase length (Beitins, McArthur, Turnbull, Skrinar, & Bullen, 1991). Daily measurement of progesterone is therefore ideal, but often impractical, to study the integrity of the luteal phase (Soules, Steiner, Clifton, & Bremner, 1984).

Part II-The Normal Hypothalamic-Pituitary-Adrenal Axis

Overview

Living organisms survive by maintaining a complex state of dynamic equilibrium or homeostasis (Chrousos & Gold, 1992). This equilibrium is constantly threatened by intrinsic or extrinsic forces or stressors. Stressors may be physical such as exercise (Stratakis & Chrousos, 1995), immobilization, cold, and hemorrage (Pacak et al., 1995). Stressors may also be psychological such as deadlines, relationships, emotional upset, and/or family concerns (Kopin, 1995). A repertoire of physical reactions or mental coping strategies are available that attempt to counteract the stressors in order to reestablish homeostasis (Chrousos & Gold, 1992). These reactions may be specific to the stressor, or generalized and nonspecific (Chrousos & Gold). The latter response only occurs if the magnitude of the threat to homeostasis exceeds a certain threshold (Chrousos & Gold). This generalized response involves the stimulation of both the HPA and the sympathetic branch of the autonomic nervous system (Chrousos, 1992).

Components of the HPA

The HPA (figure 2.3) includes the corticotrophic releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus, adrenocorticotrophic hormone (ACTH) secreted from the anterior pituitary, and cortisol, a glucocorticoid secreted from the zona fasiculata of the adrenal cortex (Chrousos, 1992; Rhoades & Pflanzer, 1992). Both ACTH and cortisol levels have been shown to increase with injections of exogenous ovine CRH (Hotta, Shibasaki, Masuda, Imaki, Demura, Ling, & Shizume, 1986). The HPA is therefore activated when CRH stimulates the anterior pituitary to secrete ACTH which in turn stimulates the adrenal gland to secrete cortisol (Chrousos and Gold, 1992).

Feedback Loops of the HPA

There are many feedback loops that regulate the HPA (Calogero, Gallucci, Gold, & Chrousos, 1988). Researchers have provided evidence for ultra short loop positive feedback of CRH in rats, where CRH secretion causes a further increase in CRH (Ono, DeCastro, & McCann, 1985). There is also long loop negative feedback in the HPA where cortisol acts to suppress both ACTH and CRH secretion (Schulte, Chrousos, Gold, Booth, Oldfield, Cutler, & Loriaux, 1985; Stratakis et al., 1995).

Circadian Rhythm

The hormones of the HPA display a circadian rhythm (Schulte et al., 1985). Cortisol levels have been shown to be highest at 8:00 in the morning, decline throughout the day to a low at midnight, then rise continuously until 8:00 a.m. (Krieger, Allen,

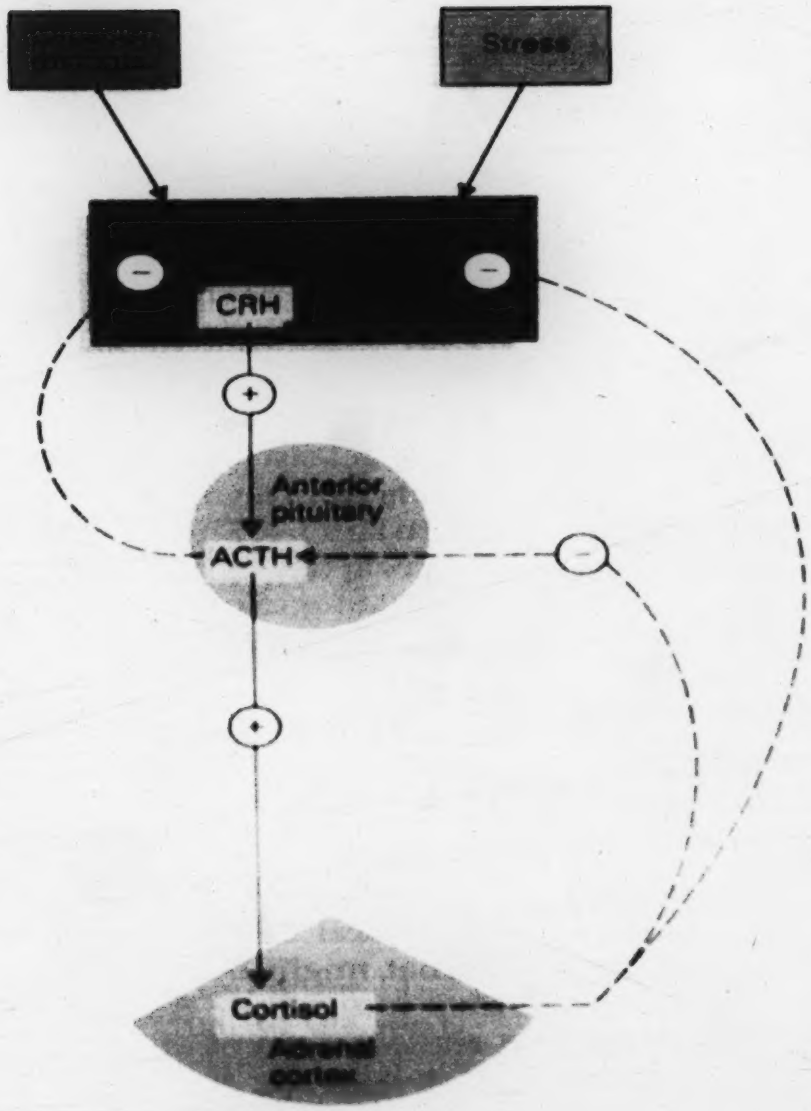


Figure 2.3 **An overview of the HPA. Stimulatory effects are shown by the solid arrows, inhibitory effects are shown by the dashed arrows. From Human Physiology (p.483) by Rhoades and Pflanzer (1992).**

Rizzo, & Krieger, 1971; Suh, Liu, Berga, Quigley, Laughlin, & Yen, 1988). There do not appear to be gender differences in this circadian rhythm (Krieger et al.). Cortisol secretion occurs in pulses (Suh et al.). In addition to CRH, factors such as arginine vasopressin (De Bold, Sheldon, DeCherney, Jackson, Alexander, Vale, Rivier, & Orth, 1984), catecholamines (Pacak et al., 1995), oxytocin, angiotensin II, (Schulte et al.) and low blood glucose levels (Rhoades & Pflanzer, 1992) may stimulate the HPA and influence the circadian rhythm of the HPA.

Physiology of the HPA

Corticotropin-releasing hormone contributes to the coordination of the physical and behavioural aspects of the stress response and regulates the immune/inflammatory reaction (Vamvakopoulos & Chrousos, 1994). Intracerebroventricular administration of CRH in rats has been associated with physiological and behavioural changes (Chrousos, 1992). These changes include increased arousal, decreased feeding and sexual activity, and "freezing" in unfamiliar environments (Chrousos & Gold, 1992). Women have a slightly exaggerated ACTH response to exogenous CRH and prolonged cortisol response when compared to men (Chrousos). Researchers have found no evidence of cortisol concentrations varying across the menstrual cycle (Stewart, Penn, Parton, Ratcliff, & London, 1993). Stewart et al. found no differences between ACTH and cortisol pulse amplitudes or frequencies and phase of the menstrual cycle. However, they did find a significant reduction in luteal phase ACTH mean levels and the area under the ACTH curve during the luteal phase (Stewart et al.). These findings may indicate changes in luteal phase cortisol binding globulin, an agonistic action of progesterone on cortisol, or

alterations in cortisol metabolism during the luteal phase (Stewart et al.). It is also possible that ACTH responsiveness to CRH is altered during the luteal phase.

Cortisol, a glucocorticoid, has many physiological actions. These include neurochemical actions, antiinflammatory actions, immunosuppressive actions, carbohydrate metabolism, and fluid balance actions (Munck, Guyre, & Holbrook, 1984). As a component of the HPA, cortisol works in conjunction with norepinephrine and the sympathetic nervous system to coordinate the stress reponse. The antiinflammatory and immunosuppressive actions of glucocorticoids seem to be in opposition to their role in the stress response since inflammation and the immune response are protective mechanisms for the body (Munck et al.). However, it has been suggested that these seemingly antithetical actions of glucocorticoids may be designed to protect the body from its own, potentially overzealous, defenses (Craddock, 1978). Glucocorticoids affect carbohydrate metabolism by inhibiting insulin secretion and by prolonging increases in blood glucose caused by glucagon and epinephrine (Munck et al.). Glucocorticoids further increase blood glucose levels by promoting hepatic gluconeogenesis and inhibiting glucose uptake in several peripheral tissues (Munck et al.). Fluid balance is also partially regulated by glucocorticoids. Antidiuretic hormone secretion is supressed and the glomerular filtration rate is increased by glucocorticoids (Munck et al.). In addition, supraphysiologic levels of glucocorticoids have been shown to cause osteopenia by decreasing bone formation and by increasing bone resorption (Hahn, Halstead, Teitelbaum, & Hahn, 1979). The mechanism for this effect is likely a result of a glucocorticoid induced rise in parathyroid hormone, rather than a direct effect of cortisol

(Baylink, 1983). Glucocorticoids have also been associated with decreased intestinal calcium absorption which may contribute to glucocorticoid-induced osteoporosis (Hahn et al.).

HPA Response to Exercise

The HPA response to exercise is variable (Viru, Karelson, & Smirnova, 1992). While cortisol levels are higher in athletic women compared to sedentary women (Ding et al., 1988; Loucks et al., 1989), the HPA response to exercise is affected by both intensity and duration of the exercise in both athletic and sedentary populations.

Viru et al. (1992) studied 50 untrained men and 32 male athletes to study the dynamic alterations in blood hormones during 2 hours of prolonged exercise. One and a half hours after a standardized breakfast, subjects were asked to cycle at 60% of their maximal power output (as determined by a prior VO_2 max test) for 2 hours. Venous blood samples were obtained in the 10^{th}, 20^{th}, 30^{th}, 60^{th}, and 120^{th} minute of exercise and 1, 6 and 24 hours after the end of the exercise. These researchers found the majority (53%) of endurance trained male athletes showed an increase in plasma cortisol after 20 minutes of exercise and 88% of endurance trained athletes showed an increase in plasma cortisol after 120 minutes of exercise. In untrained males, only after 120 minutes of exercise did the majority (56%) of the subjects show a rise in plasma cortisol levels. Both groups showed a continuous rise in ACTH throughout the 120 minutes of exercise.

Luger et al. (1987) studied the effects of physical conditioning on the HPA. Adrenocorticotrophic hormone, cortisol and lactate responses to graded levels of treadmill exercise were determined in 21 male subjects. The subjects were divided into 3

groups: sedentary (untrained, n=7), moderately trained runners (ran 24-40 km/week, n=7) and highly trained runners (ran >75km/week, n=7). Subjects were tested on 3 separate occasions and on each occasion they were asked to perform exercise of a different intensity. Each subject performed one 20 minute bout of exercise at 50% VO_2 max, one 20 minute bout at 70% VO_2 max, and one 10 minute bout at 90% VO_2 max. These researchers found that basal levels of both ACTH and cortisol were elevated in highly trained male subjects. Exercise at 50% of maximum oxygen consumption (VO_2 max) did not cause an elevation in ACTH or cortisol in trained, moderately trained, or untrained men. Exercise at 70% and 90% of VO_2 max produced proportional activation of the HPA in all 3 groups. Stimulation of the HPA was proportional to relative rather than absolute workload. Therefore, the highly trained athletes had to exercise at higher percentages of their VO_2 max to activate the HPA than their untrained peers. Lactate production was also related to relative not absolute workloads and was found to be correlated with pituitary-adrenal activation (Luger et al.). Chrousos (1992) postulates that the lactate molecule, which is produced in the exercising muscle, could be a messenger between the exercising muscle and the HPA. Lactate infusions in similar concentrations to those achieved by exercise have been shown to stimulate ACTH secretion (Chrousos, 1992). The concentration of ACTH secreted however, did not reach the concentrations seen during exercise, suggesting that lactate is only one of the many factors which influence the HPA during exercise (Chrousos). Lactate is a fuel for the working muscle and it is logical that as the exercise demands increase, both lactate production and the

HPA, which can mobilize glucose, are stimulated to meet the fuel demands of the working muscle.

Acute adrenocortical responses to exercise have also been examined in women (Cumming, Strich, Brunsting, Greenberg, Ries, Yen, & Rebar, 1981). Cumming et al. (1981) studied the acute cortisol response to incremental exercise, conducted to a symptom-limited maximum, on a cycle ergometer in regularly menstruating sedentary women (n=5), regularly menstruating runners (n=5), and amenorrheic runners (n=6). Serum cortisol levels were assessed every 15 minutes for 30 minutes before the exercise, during the exercise test and for 75 minutes following the exercise. Basal cortisol levels were significantly higher in the runners than in the sedentary women. During exercise, serum cortisol levels decreased initially in the nonrunners and then increased to a maximum following exercise. The runners lacked the initial decrease in serum cortisol but were not otherwise different from the nonrunners (Cumming et al.)

In summary, athletes generally have higher basal ACTH and cortisol levels than sedentary individuals and the HPA response to exercise is dependent on the intensity of exercise and the training of the individual.

HPA Response to Eating

Cortisol secretion also increases after eating (Suh et al., 1988). Suh et al. found a 30% increase in cortisol levels 45 minutes after eating. Ishizuka, Quigley, and Yen (1983) conducted an elaborate 5 part study to investigate the neuroendocrine relationship of food-induced pituitary hormone release as influenced by the sight and taste of food, the composition of meals, and ingestion of neurotransmitter substrates (tyrosine, tryptophan,

and choline). In addition, the potential roles of dopamine, opioid, and cholinergic systems in pituitary hormone release were investigated. The subjects in this study were 7 men aged 19-39 years. Throughout all of the experiments, blood was drawn via an intravenous (IV) catheter every 15 minutes and analyzed for prolactin and cortisol. In the first part of this study, subjects were served a breakfast and lunch, containing 600 calories which consisted of 20% protein, 40% carbohydrate, and 40% fat. In the second part of the study, each participant was served a lunch as described in the first part of the study, only without a preceding breakfast. On a separate occasion, the subjects were also served a sham lunch where they saw, smelled and chewed the meal but did not swallow it. In the third part of this study, subjects were fed a 500 calorie 455mL lunch composed primarily of one food type. Protein, carbohydrate and fat meals were served to the subjects separated by one week intervals. In the fourth part of the study, subjects ingested L-tyrosine, L-tryptophan, choline, or a lactose placebo. In the final part of the study, dopamine, naloxone, atropine sulfate or saline were administered 2 hours before the noon meal without a preceding breakfast. The results of this study showed that ingestion of a standardized meal at noon, but not at breakfast, elicited a consistent release of prolactin and cortisol. The physiological role of this noon meal associated hormone release is unclear. It has been suggested that prolactin may regulate fat stores and fat mobilization and that the effect of prolactin on fat stores is determined by the timing of the associated cortisol secretion (Ishizuka et al.). Dopamine was found to inhibit the meal-induced prolactin release whereas naloxone and choline were not involved in the food related prolactin and ACTH release (Ishizuka et al.).

Pathophysiology of Hypersecretion in the HPA

Elevated cortisol levels have been associated with many pathophysiological states which include Cushing's syndrome, anorexia nervosa, hypothalamic amenorrhea, malnutrition, polycystic ovarian syndrome, and depression (Gindoff & Ferin, 1987; Gold, Gwirtsman et al., 1986; Gold, Loriaux et al, 1986; McKenna, 1988; Stewart, 1993; Suh et al., 1988). Cushing's syndrome produces typical physiological manifestations, including altered fat deposition, muscle weakness, altered glucose metabolism, inhibition of inflammatory and immune responses, increased gastric acid secretion, hirsutism, mild acne, menstrual irregularities, and in advanced cases, thin skin, purple striae, and osteoporosis (Porth, 1990). Smith, Bledsoe, and Chhetri (1975) found that cortisol levels were higher in individuals with protein and calorie malnutrition. Hypothalamic amenorrhea has been associated with increased cortisol levels (Berga, Mortola, Girton, Laughlin, Pham, & Yen, 1989; Biller, Federoff, Koenig, & Klibanski, 1990; Suh et al.). The increase in cortisol, which is typically seen after eating, is not seen in patients with hypothalamic amenorrhea (Suh et al.). Muscle protein breakdown has been shown to be accelerated in rats with high plasma concentrations of glucocorticoids (Tomas, Munro, & Young, 1979). Cortisol levels are higher in amenorrheic versus eumenorrheic athletes and in anorexic patients vs. eumenorrheic sedentary females (Ding, Sheckter, Drinkwater, Soules, & Bremner, 1988; Glass et al., 1987; Hotta et al., 1986). It is puzzling that these groups which are chronically hypersecreting cortisol do not appear "Cushingoid". Gold, Gwirtsman et al. (1986) postulate that the absence of Cushingoid features in underweight individuals with anorexia can be explained by tissue resistance to

glucocorticoids coupled with the lack of sufficient substrate for the increased lipogenesis, fat deposition, and gluconeogenensis, characteristic of patients with Cushing's disease. Cortisol has many biological functions. Hypersecretion of this hormone may represent dysregulation of the generalized stress response (Stratakis et al., 1995).

Part III-Types of Menstrual Disorders Associated with Athletic Women

There are 5 main types of menstrual disorders that have been associated with athletic women: primary amenorrhea, delayed menarche, secondary amenorrhea, oligomenorrhea, and luteal phase defects (luteal phase shortening, luteal phase insufficiency). Potential causal mechanisms for these disorders will be discussed in chapter two, part V.

"Exercise-associated" or athletic amenorrhea is a diagnosis of exclusion (Marshall, 1994). Primary amenorrhea is the absence of menarche beyond age 16 (Loucks & Horvath, 1985). Primary amenorrhea may be associated with exercise in athletic females only when abnormalities of the reproductive tract, ovaries and pituitary have been excluded (Marshall). Both primary and secondary exercise-associated amenorrhea are generally considered to be a subset of hypothalamic amenorrhea (HA)(Marshall).

The age of menarche is affected by genetics, social factors, nutrition, and disease (Lindholm et al., 1994). Menarche has been shown to occur later in young female athletes than in their sedentary peers (Malina, 1983; Lindholm et al.; Loucks, 1990; Ronkainen et al., 1984). In the United States, the mean age of menarche is 12.5-12.9 years (Greene, 1993; Loucks; Warren, 1992). In 1980, an unspecified group of

synchronized swimmers had a mean age of menarche of 13.0 years (Ross & Corlett, 1980 in Malina, 1983). Generally, athletes who begin training before menarche begin menstruating later and have a higher incidence of menstrual disorders than athletes who begin training after menarche (Guler & Hascelik, 1993; Lindholm et al.; Loucks). Loucks et al. (1992) postulate that exercise and undernutrition may have profound effects on the timing of puberty, the release of gonadotropins, and the onset of menstrual disorders in female athletes. However, they caution that although menarche occurs later in athletes than non athletes, it remains to be demonstrated that exercise causes delayed menarche (Loucks et al.).

Abnormal LH pulse patterns have been associated with hypothalamic amenorrhea (Reame, Sauder, Case, Kelch, & Marshall, 1985), exercise trained females (Cumming, Vickovik, Wall, & Fluker, 1985; Warren, 1992), and luteal phase defects (Schweiger et al.,1989; Soules et al., 1984; Suh & Betz, 1993). Hypothalamic amenorrhea (HA) is a menstrual disorder which is diagnosed when abnormalities in pituitary and ovarian function cannot be identified (Reame et al.). Changes in the GnRH pulse pattern are likely the cause of secondary HA (Remorgida, Venturini, Anserini, Salerno, & De Cecco, 1990). Reame et al. found that women with HA had lower LH, and by inference GnRH pulse frequencies than eumenorrheic women yet normal LH pulse amplitudes. Loucks et al. (1989) studied 24-hour patterns of pulsatile LH secretion in 26 women. The subjects in this study were divided into 3 groups: eumenorrheic athletes (n=9), amenorrheic athletes (n=9), and sedentary women (n=8). The amenorrheic athletes had the fewest LH pulses but normal pulse amplitude (Loucks et al.). Veldhuis, Evans, Demers, Thorner,

Wakat, and Rogol (1985) also reported decreased LH pulse frequency in some long-distance runners with secondary amenorrhea. These researchers, like Loucks et al., did not find an associated decrease in spontaneous LH pulse amplitude (Veldhuis et al.). Amenorrheic athletes, therefore, tend to have normal LH pulse amplitude but decreased LH pulse frequency when compared to their sedentary peers.

In athletic women, changes in hormone concentrations and cycle length may occur despite regular menses. Cumming, Vickovik, Wall, and Fluker (1985) found a decreased early follicular phase LH pulse frequency in normally menstruating runners when compared to sedentary controls. Loucks et al. (1989) also found decreased early follicular phase LH pulse frequency but increased pulse amplitude in regularly menstruating runners. Pirke, Schweiger, Brooks, Tuschl, and Laessle (1990) demonstrated that athletes with normal menstrual cycles had reduced E_2 and P secretion and shorter menstrual cycles than sedentary controls despite normal LH and FSH pulsatility.

Oligomenorrhea (menstrual cycles occurring at intervals from 39-90 days) (Loucks & Horvath, 1985) has also been reported in athletic women (Erdelyi, 1976; Greene, 1993; Loucks & Horvath; Russell et al., 1984a). Studies have reported oligomenorrhea in female athletes during their training seasons with regular menses returning during the off-season or during times of less strenuous training (Erdelyi; Russell et al.).

Defective luteal phases are greater than 9 days but have inadequacies in the production and/or biological effects of progesterone (Beitins et al., 1991). Shortened

luteal phases are those which are less than 9 days (Beitins et al.). Soules et al. (1984) studied LH pulsatility in women with luteal phase deficiency and discovered that these women had higher LH pulse frequency in the early follicular phase of their cycle. Two possible explanations for this increased pulse frequency were postulated: 1) since LH pulse frequency is normally diminished in the luteal phase as a result of the increased P concentrations at that time, a decrease in P in the previous cycle may not suppress LH pulsatility in the subsequent follicular phase, and 2) a defective pulse generator may cause an increase in LH pulse frequency (Soules et al., 1984). The increase in early follicular phase LH pulse frequency observed by Soules et al. are in contrast to the findings of Schweiger et al. (1989) and Schweiger, Tuschl, Brooks, and Pirke, (1990), who found a decrease in early follicular phase LH pulsatility associated with luteal phase abnormalities. Pirke et al. (1990) reported high variability in the LH pulse secretion in athletes with luteal phase abnormalities. The prevalence of luteal phase defects is also difficult to quantify since these disorders occur in women who often report regular menses. Vuorento, Lahti, Hovatta, and Huhtaniemi (1989) found that 47 % of the 17 university students (aged 20-31 years), who reported regular menstrual cycles, had insufficient or shortened luteal phase cycles.

In summary, both HA and luteal phase defects are associated with alterations in LH pulsatility in the early follicular phase of the menstrual cycle. Since LH pulses are a direct result of stimulation by GnRH (Knobil, 1980), the cause of these menstrual disorders is likely related to the GnRH pulse generator. Further support for this line of reasoning comes from work by Loucks et al. (1989) and Veldhuis et al. (1985) who did

studies giving a bolus injection of exogenous GnRH to amenorrheic runners. They found that the pituitary response to GnRH was exaggerated in the runners versus sedentary controls (Loucks et al.; Veldhuis et al.). These data indicate that the source of LH inhibition is at the hypothalamus or in the regions which modulate hypothalamic activity (De Souza & Metzger, 1991). The potential mechanisms for alterations in GnRH and subsequently LH pulsatility will be discussed later.

Part IV- Measurement Issues Associated with Menstrual Disorders

There are many internal and external factors associated with menstrual disorders. Internal factors include: hormone levels, gynecologic history, menstrual cycle characteristics, and body fatness. External factors include: exercise training, energy intake, stress, and season. There has been conflicting evidence associated with many of these factors in the literature. This conflict is likely due to the fact that monitoring many of the factors associated with menstrual disorders is difficult. Menstrual cycles vary between individuals and within the same individual. Assessment of many of the variables associated with menstrual disorders must be controlled for the day of the cycle and time of day. It is difficult to compare many of the conflicting results since many researchers do not clearly define their methodology or clearly describe their subjects. Furthermore, many of the best methods are invasive and expensive, making them prohibitive to many researchers.

The following section will discuss methodological issues which should be considered when researching menstrual disorders and the strengths and limitations of the

methods reported in the literature. The link that each of these factors has with reproduction will be discussed in Part V of this review.

Prevalence

The reported incidence of menstrual disorders in athletic women varies widely in the literature. Ranges of 3.4-66% (Nattiv et al., 1994) have been reported. This wide variation can likely be explained by a lack of standardized definitions for menstrual disorders and the wide variety of research techniques used to estimate prevalence rates (De Souza & Metzger, 1991).

Prospective vs. Cross Sectional Studies

Cross sectional designs permit greater subject participation in time consuming hormonal studies. However, cross sectional designs often fail to control for the intra-individual variation which may occur independently of any experimental manipulations. Cross sectional studies and surveys are frequently unable to control for lifestyle variables other than exercise which may disturb menstrual function (Loucks, 1990).

Few prospective studies on the relationship between exercise and menstrual disorders have been done. Three studies have implemented exercise training programs in previously untrained individual to investigate the relationship between exercise and menstrual disorders (Beitins et al., 1991; Bonen, 1992; Bullen et al., 1985). The studies conducted by Beitins et al. and Bullen et al. involved similar exercise protocols and rather sudden onset of training (no previous training to 6.4 km per day, 5 days per week). The study by Bonen had a gradual onset of exercise but was poorly controlled for exercise intensity.

Others have investigated menstrual disorders in trained athletes. A study by Russell et al. (1984b) followed competitive swimmers for 2 years. The swimmers served as their own control group and were followed during cycles of varying training volumes and intensities to record the incidence of menstrual irregularities in this group (Russell et al.). Blood sampling in this study was infrequent (2 serum samples in total) and poorly controlled for varying menstrual cycle length and for menstrual cycle phase (Russell et al.).

Despite these methodological flaws, prospective designs allow the researcher to determine baseline hormonal values for each subject and to control for other predisposing factors which are suspected to be associated with the onset of menstrual disorders. Independent manipulation of exercise (type, amount, intensity, and rate of onset) and energy (caloric intake, energy expenditure, and energy deficit) are possible in prospective studies, and necessary to assess the influence of an athletic lifestyle on female menstrual function (Loucks, 1990).

Season

Season has been shown to be another factor to consider when studying menstrual disorders. Ronkainen et al. (1985) studied the effect of the varying luminosity in northern Finland on the menstrual cycles of runners and nonathletic women. They found that ovarian hormone levels were lower in the fall (darker photoperiod) compared to that in the spring (lighter photoperiod) whereas anterior pituitary function did not vary (Ronkainen et al.). This phenomenon was exercise independent (Ronkainen et al.) and

underscores the importance of a control group in longitudinal studies which may incorporate photoperiods of varying length.

Hormone Levels

The measurement of hormones in females is difficult because of the pulsatile, cyclic, and rhythmic nature of most hormones. In females, hormonal testing must be controlled so that each subject is studied at the same time in the menstrual cycle, when the hormonal milieu is similar. Blood sampling has been poorly controlled in the literature for menstrual cycle phase (Loucks & Horvath, 1985; Bonen, Haynes, & Graham, 1991). Even when hormonal testing times are controlled for the phase of the menstrual cycle, there are significant inter-individual variations in resting hormone levels (Hall-Jurkowski, 1982). Hormonal pulse patterns are unique to each individual. Values are therefore often reported as means to minimize the effect of individual variability (Bonen, 1992; Rogol et al., 1992). When studying menstrual disorders, this practice may be dangerous, as valuable outlying data may be lost. Intra-individual hormonal variations from one cycle to the next are also common and may confound the results of longitudinal studies.

Biochemical Analysis

Hormone analysis performed using radioimmunoassays should be controlled for both intraassay and interassay variation. Samples for one subject at all testing times should be analyzed using the same assay kit at the same time. This minimizes the interassay variation. Intraassay variation is minimized by placing known control samples intermittently throughout an assay to ensure that the experimental conditions are similar

from the start of an experiment to the end. Acceptable values for between duplicate coefficients of variation (CV) are 6-15% (Chard, 1987). Variations between assays should be considered when comparing results from one study to another; interassay variation may account for the observed differences in hormonal levels (De Souza & Metzger, 1991).

Hormone Pulse Detection

Hormone pulse detection methods are variable (Murdoch, Diggle, Dunlop, & Kendall-Taylor, 1985; Veldhuis & Johnson, 1986). Visual identification of hormone pulses is possible but is subjective and may not be reproducible between researchers (Veldhuis & Johnson). Many computer programs have also been used to identify endocrine pulses. This method is more objective, however numerous methodologies have been used and a wide variety of pulse parameters have been reported (Veldhuis & Johnson). Veldhuis and Johnson list 7 limitations of hormone pulse analysis: 1) inconsistent pulse frequency estimates due to fluctuating baseline hormone concentrations, 2) requiring relatively uniform pulse amplitudes within a given series of samples, 3) the use of a single point to define an increase as a peak, 4) the lack of a consistent definition for the downslope of a peak, 5) the dependence on assay procedures which are independent of the samples, 6) the potential for intra-assay variation, and 7) the absence of a clear statistical basis for hormone pulse analysis. These researchers therefore recommend a cluster analysis program to analyze LH pulses in order to improve pulse detection consistency, to increase control over desired pulse parameters (peaks and nadirs), and to minimize the false-positive rate of pulse detection (Veldhuis & Johnson).

This method has been widely adopted in the literature and has led to some consistency in results between researchers. However, even among studies that use the Veldhuis and Johnson cluster analysis algorithm for endocrine pulse detection, there is variation in the t-statistic and minimum size of nadir and peak widths (cluster size) chosen (Judd et al., 1995; Suh et al., 1988). This variability can be attributed to specific research design (sampling frequency, population studied, hormone investigated), efforts to minimize false-positive error rates in pulse detection by increasing the t-statistic, and/or efforts to optimize the sensitivity of pulse detection (Veldhuis & Johnson).

Measurement of Hypothalamic and Pituitary Hormones

Pituitary and hypothalamic hormones are particularly difficult to measure because of their episodic secretion and variable plasma half lives (Yen, Tsai, Naftolin, Vandenberg, & Ajabor, 1972). Due to the low peripheral concentrations of GnRH and the short half life of this hormone (2-4 minutes), LH is often sampled in the periphery to reflect GnRH secretion (Crowley et al., 1985). This sampling is based on 2 assumptions: 1) that only GnRH is capable of causing gonadotropin secretion and 2) that all GnRH pulses cause gonadotropin secretion (Crowley et al.). Blood sampling is often not frequent enough to detect the discrete nature of endocrine pulses (Loucks & Horvath, 1985; Veldhuis & Johnson, 1986). Crowley et al. report that optimum luteinizing hormone (LH) sampling is at 5-10 minute intervals. This time interval between samples allows visualization of all major GnRH pulses (Crowley et al.). Due to practical blood sampling limitations, 10 minute sampling intervals are often used (Crowley et al.). Although this time interval is acceptable, there is a risk of underestimating GnRH

secretion when pulse frequency is high or pulse amplitude is low (as in hypogonadotropic disorders) (Crowley et al.). In LH measurement, the duration of this serial blood sampling is important. Early follicular phase LH pulses occur approximately every 90 minutes (Crowley et al.). Therefore, the duration of sampling must be sufficient to observe several potential secretory episodes. Studies have shown that LH pulse frequency slows and pulse amplitude is diminished in cyclic women during sleep (Crowley et al.; Loucks et al., 1989), whereas amenorrheic women showed no sleep related changes in LH secretion (Loucks et al.). Subjects must therefore be encouraged to remain awake during daytime sampling to ensure an accurate assessment of LH pulses. Activation of the HPA has been shown to lower plasma LH levels in both rat (Rivier & Vale, 1984) and human (Judd et al., 1995) models. The acute stress of the insertion of the indwelling intravenous catheter necessary for serial blood sampling may briefly inhibit LH pulsatility . Subjects should, therefore, sit for a minimum of 30 minutes after insertion of the indwelling catheter before sampling is started.

Weltman, Veldhuis, Weltman, Kerrigan, Evans, and Rogol (1990) examined the statistical reliability of specific characteristics of pulsatile LH secretion during the early follicular phase of 2 separate menstrual cycles in eumenorrheic women. They found that the LH concentrations over 24 hours were reproducible assuming a stable within-subject hormonal half life, when studied serially in the same woman at the same time in the menstrual cycle. However, considerable variations in some of the pulsatile patterns, which generate the integrated LH concentration, were observed (Weltman et al.).

Measurement of Progesterone

Accurate biochemical assessment of the luteal phase of the menstrual cycle requires daily sampling for P (Zorn, McDonough, Nessman, Janssens, & Cedard, 1984). Daily blood samples are often impractical and inconvenient for subjects. Daily salivary progesterone samples have been shown to be highly correlated with plasma progesterone and are therefore a non-invasive alternative to daily blood samples (Vuorento et al., 1989; Zorn et al.).

Measurement of Cortisol

Cortisol secretion is not affected by menstrual cycle phase (Stewart et al., 1993), however cortisol has a characteristic circadian rhythm (Krieger et al., 1971; Schulte et al., 1985; Suh et al., 1988). Measurement techniques for this hormone must therefore account for the daily variations in cortisol concentrations (Thuma, Gilders, Verdun, & Loucks, 1995). 24-hour urine collection is a non-invasive measurement technique which quantifies cortisol levels in the urine and accounts for the circadian rhythm of this hormone.

Gynecologic History

A woman's gynecologic history is an important factor when studying menstrual disorders. Previous pregnancy and reproductive maturity may protect against the development of menstrual disorders, while reproductive immaturity may be a predisposing factor to menstrual disorders (Loucks & Horvath, 1985). Gynecologic maturity is also a major consideration when young women are studied. Metcalf, Skidmore, Lowry, and Mackenzie (1983) found that 5 years after menarche, 83.3% of

students studied had ovulatory cycles. This is in contrast to the 44.6% of females whose cycles were ovulatory in the first year of menstruation (Metcalf et al.). These researchers found that the incidence of ovulatory cycles increased with gynecologic age (Metcalf et al.).

Menstrual Cycle Characteristics

Cycle length is a common measure used to assess menstrual function. However, a lack of universally accepted definitions of various menstrual patterns has led to a wide variety of criteria used to assess menstrual cycle pathology (Loucks & Horvath, 1985). The duration of each cycle is not frequently reported and the amount of menstrual blood lost is difficult to quantify. Higham et al. (1990) compared menstrual blood loss measurements (in mL) to scores obtained from pictorial blood loss assessment charts (PBAC) which account for both the number of pads or tampons used and the degree to which they are soiled. They found a positive correlation ($r = 0.74$) between the total number of sanitary pads and tampons used and menstrual blood loss (in mL) (Higham et al.). This simple chart, therefore, complements cycle length and menstrual flow duration information and adds to the objective characterization of subjects' menstrual cycle characteristics.

Body Fatness

The measurement of body fatness is an inexact science (Lohman, 1992). All methods are predictive and are therefore associated with both biological and measurement error. Furthermore, many of the standards used are not specific for athletic populations (Loucks & Horvath, 1985).

Historically, hydrostatic weighing has been the criterion measure for body fatness, yet is rarely used in research (Loucks & Horvath, 1985). Hydrostatic weighing uses Archimedes' principle of water displacement to measure body volume. From body volume, body density can be determined. In hydrostatic weighing, density is determined from an assumed 2 compartment model, consisting of fat mass (FM) and fat free mass (FFM) (Withers, Smith, Chatterton, Schultz, & Gaffney, 1992). FM consists of both essential and non-essential fat. FM is assusmed to be anhydrous, potassium free, and to have a constant density. FFM is composed of water, protein, bone mineral, and non-bone mineral. The assumed density of the FM has not been challenged (Withers et al.). The assumed constant density of the FFM is problematic. The water and bone mineral components of FFM cause the most variability in body density estimations (Martin & Drinkwater, 1991; Snead, Birge, & Kohrt, 1993; Withers et al.). Therefore, hydrostatic weighing may not provide an accurate assessment of body fat in athletes with menstrual disorders, since their potentially reduced bone density may lead to an overestimation of body fatness (Highet, 1989; Martin & Drinkwater, 1991).

More recently, computed tomography (CT) scanning, nuclear magnetic resonance imaging (MRI), and dual-energy x-ray absorptiometry (DEXA) have challenged hydrostatic weighing as the criterion measure for body composition. Computed tomography scanning produces a cross sectional image and can be used to validate existing anthropometric techniques or to provide an understanding of the relationship between metabolic activity and body composition (Brodie, 1988). This technology may also be used to detect fat distribution (Brodie). Ethically, CT scanning may not be a

preferred body composition assessment method since it involves exposure to radiation (Brodie). Nuclear MRI requires a bore magnet large enough to accept the human body. The images produced are received by a radio frequency coil and are dependent on the behaviour of the hydrogen nuclei in the magnetic field (Brodie). Magnetic resonance imaging and CT scanning technology are accurate, yet expensive (Brodie). Due to a limited number of CT and MRI machines, scheduling time for research on these machines is also problematic. Dual-energy x-ray absorptiometry uses a stable x-ray generator and 2 energy levels as the radiation source to assess body composition (Hansen, Lohman, Going, Hall, Pamenter, Bare, Bayden, & Houtkooper, 1993). A whole body DEXA scan takes approximately 10 minutes and exposes the participant to approximately 0.05 mrem of radiation (Hansen et al.). This method is based on a 3 compartment model of body composition (Withers et al., 1992). The 3 compartments are: bone mineral content (BMC), fat mass, and lean tissue mass. This model is superior to the 2 compartment model in that one aspect of FFM, BMC, is quantitatively assessed. Dual-energy x-ray absorptiometry has been validated against bone ash in excised skeletons, bone ash *in vivo*, and extracted fat from *in vitro* phantoms (Withers et al.). As with hydrodensitometry, there are also limitations to DEXA. First, only 1 of the 4 components of FFM is measured. Therefore, there is still the potential for variability from the assumed value in the non-bone mineral, protein, and water components of FFM. As with hydrodensitometry, DEXA assumes that FFM of men and women have identical water content. In addition, DEXA may underestimate fat in the trunk region (Snead et al.,

1993). Although this method is an accurate measure of body composition, it is expensive and more difficult to access than many other methods.

As an alternative, skinfold measurements indicate fat distribution, fat loss, and overall adiposity (Brodie, 1988; Marshall, 1989) and are a valid method for body composition assessment (Jackson & Pollock, 1985). Potential sources of error in skinfold measurement are caliper selection and tester reliability (Pollock & Jackson, 1984). Harpenden and Lange calipers have been compared and have both been shown to exert constant pressure and produce accurate results (Pollock & Jackson). The validity of skinfold measurements is related to both inter- and intra-tester errors in measurement (Pollock & Jackson). Intra-tester error can be minimized with sufficient training and by taking multiple measurements of each site (Jackson & Pollock; Pollock & Jackson). Inter-tester error can be minimized in research by having the same tester measure all subjects whenever possible. Selection of skinfold sites is another potential source of error which can be minimized with accurate landmarking and marking the sites to be measured (Pollock & Jackson). Regression equations used to determine percent fat may increase the error associated with skinfold measurement since selecting an appropriate equation adds one more variable which may impede the accurate assessment of body fatness.

Training

When studying the effect of athletic training on the menstrual cycle, an accurate quantification of training frequency, duration, intensity, and volume is required. Many studies have used recall as a method of quantifying training. This is problematic because subjects are not likely to accurately recall their years of previous training, the training

volumes or the hours per week of training. In addition, the training background and the training intensity of subjects are often inconsistent or not documented (Loucks & Horvath, 1985). Many studies also fail to specify whether menstrual disorders were present before the training regimen was initiated (Loucks & Horvath). Loucks and Horvath suggest that race performance, volume, duration, intensity, frequency of the training sessions, length of the training season, and maximum oxygen consumption should all be determined and reported.

Energy Intake

Quantifying energy intake is difficult since no standard method has been found (Howat, Mohan, Champagne, Monlezun, Wozniak, & Bray, 1994). Day to day variations in food intake, memory lapses, inadequate knowledge of food portions, overestimation or underestimation of portions consumed, and the extent to which the recording methods chosen represent usual energy intake habits may affect energy intake measures (Howat et al., 1994). There are 4 main methods used to assess energy intake. These methods are: 24-hour energy recall, diet histories, food frequency questionnaires, and food records (Freudenheim, 1993; Sempos, Flegal, Johnson, Loria, Woteki, & Briefel, 1993). The strengths and weaknesses of each method will be briefly discussed.

A 24-hour energy recall involves the participant listing the foods that they have ingested in the preceding 24 hours (Freudenheim, 1993). Multiple 24-hour recalls may be used over a period of time to more fully assess intake (Freudenheim). The advantages of this method are that it is relatively fast and that it does not require the respondent to judge if the past 24-hours was typical, it only requires short-term memory (Freudenheim).

Errors in this short-term memory are the main disadvantage of 24-hour recalls (Freudenheim). Respondents may forget foods that they ate or even report foods that they did not eat (Freudenheim). Age, socioeconomic status, or health status may also influence the reliability of respondents' recall (Freudenheim). Furthermore, day-to-day variations in eating patterns make a single 24-hour recall inadequate to assess an individual's usual diet (Freudenheim).

Diet histories focus on usual energy intake (Freudenheim, 1993). Respondents are asked open ended questions about their usual food intake (Freudenheim). Portion sizes may be assessed with the use of food models (Freudenheim). Cross-check questions may be asked to avoid missing any foods (Freudenheim). Well-conducted diet histories can provide qualitative assessment of usual food intake (Freudenheim). These interviews may address current or past food intake (Freudenheim). In addition, food preparation, recipes and seasonality may be evaluated (Freudenheim). One disadvantage to this method is that the interviews can be time consuming (Freudenheim). There is also a burden on the respondents to evaluate their usual energy intake over time in order to provide an accurate summary (Freudenheim). Memory errors and the respondent's idea of a good diet may contribute to the inaccuracy of this method (Freudenheim). However, diet histories are a relatively valid source of energy intake assessment and are often used as a standard by which other methods are validated (Freudenheim).

Similar to the diet history, food frequency questionnaires assess usual intake (Freudenheim, 1993). Respondents respond to questions regarding the number of times that they consume each food from a list of foods (Freudenheim). Food lists may vary

considerably and this type of questionnaire may fail to address portion sizes, food preparation, and seasonality of food consumption (Freudenheim). Disadvantages to this method include: omissions in the food list that may make up a significant portion of the respondents' diet, errors in memory, and the respondents' bias toward what they believe constitutes a good diet (Freudenheim).

Food records involve respondents recording their intake as they eat (Freudenheim, 1993). There is the potential for considerable variation in food records. Details of portion sizes, food preparation, ingredients in mixed dishes, and restaurant meals are all potential sources of variation. The number of records required for an accurate assessment of current, usual intake depends on the nutrients to be quantified (Freudenheim). Nutrients with a large intraindividual variation like vitamin A require several records to estimate usual intake (Freudenheim). The number of days foods are recorded also depends on the nutrients to be quantified. Where 7-day records may be required to accurately estimate typical micronutrient intake, 3-day food records which include 2 weekdays and 1 weekend day have been shown to estimate habitual energy intake of calories to within 10 % of actual values (Basiotis, Welch, Cronin, Kelsay, & Mertz, 1987). Food records which are given with detailed instruction and compliant respondents are reproducible and reliable measure of energy intake (Howat et al., 1994), however there are several disadvantages to this method. First, there is a burden to keep accurate records placed on the respondents (Freudenheim). There is also the potential that respondents may alter their diet to simplify the records or alter their diets based on their perception of acceptable diets (Freudenheim). Finally, the respondents may not

record their intake as they eat, but may fill out records once per day or even less frequently (Freudenheim). In this instance, food records become prone to the memory problems associated with other energy intake assessment methods (Freudenheim).

When choosing the energy intake assessment tool to use in a given project, there are 2 factors to consider (Sempos et al., 1993). First, the researcher must consider whether the relative ranking of each individual's intake or whether an individual's absolute usual intake is desired (Sempos et al.). Second, the researcher must decide if the the objective is to estimate the intake of broad classes of food, or to evaluate more complex aspects of diet related to absorption and metabolism (Sempos et al.). Sempos et al. caution that none of the methods described above are likely to measure the true absolute energy intake for an individual. All of the methods described are associated with measurement error and the choice of a research method should be made based on assessed relative validity (Sempos et al.).

Stress

Both psychological and physical stress have been associated with menstrual disorders (De Souza & Metzger, 1991; Whitacre & Barrera, 1944). Each type of stress activates the HPA (Stratakis et al., 1995). It is therefore difficult in athletes to separate the psychological stress of competition from the physical stress of training and competing. Cortisol secretion has been shown to be stimulated by both psychological and physical stressors (Marin, Darin, Amemiya, Andersson, Jern, & Bjorntorp, 1992; Stratakis & Chrousos, 1995). Questionnaires have been shown to be reliable and valid measures of psychological stress (Derogatis, 1987; Soukup, Beiler, & Terrell, 1990;

Troop, Holbrey, Trowler, & Treasure, 1994). Used in combination, cortisol secretion and psychological stress questionnaires may provide an indication of the total stress that an individual is facing.

The Derogatis Stress Profile (DSP) is a 77 item self-report inventory designed to measure stress (Derogatis, 1987). The DSP was derived from the interactional theory of stress and has 3 principal domains: environmental events, personality mediators, and emotional responses (Derogatis, 1987). The DSP measures 11 dimensions of stress (time pressure, driven behaviour, attitude posture, relaxation potential, role definition, vocational environment, domestic environment, health posture, hostility, anxiety, and depression) which each represent one of the 3 domains (Derogatis, 1987). From the scores of the DSP, the researcher is able to calculate total stress scores and subjective stress scores for each subject (Derogatis, 1987). Highly stressed individuals should have high DSP scores. Derogatis (1995) reports that the DSP is valid and reliable as a screening or outcome measure of stress. Normative data are available for the DSP which allows researchers to compare the relative standing of a subject to other subjects of similar age and other demographic variables (Derogatis, 1987). The test-retest reliability of the DSP ranges from 0.72 for hostility to 0.92 for time pressure as determined from 34 individuals who presented to a corporate medical office with stress related disorders (Derogatis, 1987). Internal consistency coefficients, which were determined from 847 individuals who were employees of approximately 12 different major corporations, range from 0.79-0.93 for the sub-scales and from 0.83-0.88 for the 3 principle stress components (Derogatis, 1987). The DSP has been validated in a group of 66 women

aged 20-29 and data suggest that the DSP is sensitive to a broad spectrum of stress phenomena (Derogatis, 1987).

The inability to cope with stress may explain the persistence of stress despite a relative infrequency of undesirable life events (Soukup et al., 1990). How individuals cope with stress rather than stress itself may be a profound influence on their psychological well-being, social functioning, and somatic health (Folkman & Lazarus, 1988). Coping strategies such as problem solving and seeking social support are associated with lower stress levels while avoidance and denial are associated with higher stress levels (Troop et al., 1994). The Ways of Coping Questionnaire (WOC) is a 66-item questionnaire which uses a 4-point Likert scale (Folkman & Lazarus). The WOC categorizes the way that an individual has coped with a stressful life event in the past week. There are 8 sub-scales for this questionnaire: 1) confrontation coping, 2) distancing, 3) self-controlling, 4) seeking social support, 5) accepting responsibility, 6) escape-avoidance, 7) planful problem solving, and 8) positive reappraisal. Raw scores for each subscale describe coping effort for each coping type, whereas relative scores describe the proportion of effort represented by each type of coping (Folkman & Lazarus). The internal reliability of the WOC has been estimated with Cronbach's alpha, and ranges from 0.61 for distancing to 0.79 for positive reappraisal as averaged from the scores of 150 individuals tested on 5 occasions (Folkman & Lazarus). The WOC has been validated in 90 women aged 17-45 (Troop et al., 1994). Preliminary work has shown that this questionnaire has both face and construct validity (Folkman & Lazarus). The WOC has been used in longitudinal studies (Folkman, 1997). This questionnaire may help to

separate highly stressed eumenorrheic individuals from highly stressed athletes with menstrual disorders. One difference between these groups may be that one has appropriate coping skills, while the other does not.

Using the DSP and the WOC in combination may give an indication of the amount of stress an individual feels and how that individual copes with stress. It may then be possible to explain why individuals with similar nutrition, body fatness, and training may have different menstrual function.

Part V-Potential Causal Mechanisms of Menstrual Disorders

Despite measurement difficulties associated with the study of menstrual disorders, there have been many studies which have attempted to elucidate the potential causal mechanism(s) of menstrual disorders. Most studies have attempted to link the predisposing factors often seen in amenorrheic athletes: low body fatness (Frisch, 1987), high volume and intensity of physical activity (Beitins et al., 1991; Bullen et al., 1985; McArthur, Gilbert, Henery, Quinn, Perry, Cramer, Kirkland, Tunstall Pedoe, Rees, Besser, & Turnbull, 1990), high cortisol levels (De Souza et al., 1991; De Souza et al., 1994; Ding et al., 1988), and low energy availability (Wade et al., 1996), to a causal mechanism. However, an exact causal mechanism for menstrual disorders in female athletes has not been identified.

The following review will discuss potential causal mechanisms for menstrual disorders and will assume 3 main categories of predisposing factors which are associated with menstrual disorders (see Figure 1.1 for an overview of these factors): 1) energetic challenges (energy intake, body fatness and exercise training), 2) stress (psychological

and physical), and 3) other (endogenous opioid secretion, neurotransmitter secretion, menstrual history, undetermined factors). These categories are not mutually exclusive and may combine or interact to cause menstrual disorders.

Energetic Challenges

Menstrual disorders are likely not caused by one single factor, but a combination of many factors. The energy drain theory is based on energy balance. An energy drain is created when caloric intake is insufficient for the amount of energy being expended by exercise training (Loucks, et al., 1992). The energy drain theory attempts to encompass the interaction between nutrient intake, body weight, body fatness, metabolic rate, and follicular development. This theory parallels the high incidence of menstrual disorders in lean, athletic women.

Kaiserauer, Snyder, Sleeper, and Zierath (1989) studied the nutritional status of 8 amenorrheic runners (AR)(mean age 27$\pm$ 2 years), 9 regularly menstruating runners (RM)(mean age 22$\pm$3 years), and 7 regularly menstruating sedentary controls (RMSC)(mean age 22$\pm$1years). The AR and RMSC groups had significantly lower intake of many macro and micronutrients than the RM as measured by a 3-day energy intake record. Although the AR and the RM were expending the same amount of energy in training, the AR consumed fewer calories, creating a possible metabolic deficit. The findings in this study showed that inadequate nutrition and/or an energy imbalance coupled with intense exercise contribute to athletic amenorrhea (Kaiserauer et al.).

Laughlin and Yen (1996) found that 3 groups of subjects (amenorrheic athletes (AA), eumenorrheic athletes (EA), and sedentary controls(SC)) had similar caloric intake

as assessed by a 7-day energy intake record. However, these researchers found that the athletes in their study consumed less fat and protein, and more carbohydrates and fiber than the sedentary controls. When expressed as a percentage of nutrient intake, the AA diet was 13.2 ± 1.7% fat, 70.2 ± 3.4% carbohydrate, and 11.8 ± 0.9% protein; the EA diet was 24.4 ± 2.8% fat, 53.8 ± 4.8% carbohydrate, and 12.4 ± 1.0% protein; and the SC diet was 31.6 ± 1.9% fat, 51.6 ± 4.1% carbohydrate, and 17.5 ± 1.7% protein. The type of energy, in addition to number of calories, may be important when studying energy balance in athletes. The energy drain theory needs to be validated with studies which measure energy intake, energy expenditure and resting metabolic rate.

Energy Intake

If energy availability is insufficient, the body priorizes energy needs, sustaining energy for thermoregulation, cellular maintenance, and locomotion at the expense of energy for reproduction, growth, and fat storage (Wade et al., 1996). Women on marginal nourishment with corresponding low energy stores are more likely to be sensitive to even minor modifications in energy balance (Rosetta, 1993). Rosetta (1993) suggests that fasting may inhibit the GnRH pulse generator unless sufficient energy stores are present.

Cameron, Helmreich, and Schreihofer (1993) supported this hypothesis with their work on male rhesus monkeys. They found a significant slowing of LH pulsatility as little as 4 hours after fasting was initiated. They hypothesized that a 'signal' occurs very early during periods of fasting to suppress the central drive of the HPO. In states of

chronic undernutrition, an amplification of the 'signal' may occur or, additional signals also capable of suppressing the HPO may be recruited (Cameron et al.).

In addition to fasting, food restriction may also suppress GnRH pulses. Bronson (1986) conducted a study in food-restricted prepubertal female rats. The rats were maintained at 45% of their expected 50 day body weight. Indwelling atrial cannulae were implanted 48 hours prior to blood collection. Blood was collected every 5 or 6 minutes over a 3.5 hour period. LH pulsatility was measured in the food-restricted state, 12 hours after *ad lib* feeding in one group, and 24 hours after *ad lib* feeding in another group (Bronson). No LH pulses were discernable in the food-restricted state. After 12 hours of re-feeding, LH pulses were detected in 3 of 6 female rats, and after 24 hours of refeeding, 8 of 10 rats had pulsatile LH release and 6 of those rats had LH pulse patterns that were indistinguishable from those in normally fed rats (Bronson). It is interesting that LH pulsatility was induced after 12-24 hours of refeeding in these rats, and that ovulation occurred within 2.5-3.5 days of refeeding as determined by the position of the eggs in the oviduct upon autopsy. In this same experiment, another group of food-restricted rats were infused with GnRH once every hour for 3 or 6 days. The rats autopsied after 3 days of GnRH infusion had not ovulated, yet their uteri had increased in weight 4 fold from uninfused controls. All 6 rats autopsied on the sixth day of GnRH infusion had ovulated. The author notes that GnRH infusion took 1-2 days longer to induce ovulation than *ad lib* feeding. From this study, the author suggests that the mechanism responsible for reproductive disorders induced by food-restriction must reside in the neural control of GnRH secretion (Bronson).

In humans, changes in P and LH have been observed in women placed on a vegetarian 800 kcal diet for one cycle as compared to a control cycle before dieting (Pirke, Schweiger, Strowitzki, Tuschl, Laessle, Broocks, Huber, & Middendorf, 1989). All subjects (n=13) in this study had ovulatory cycles before dieting. Blood was sampled daily during both the control and diet cycles. Between the third and fifth day of each cycle, an indwelling catheter was inserted and blood samples were taken every 10 minutes for 6 hours beginning at 6:00 p.m. This 6 hour sampling was repeated at weekly intervals throughout the control and diet cycles. During the diet cycle, 7 women had anovulatory cycles, 4 women had impaired P secretion during the luteal phase after apparently normal follicular development, and average LH concentrations and number of LH pulses were reduced in the follicular phase of the diet cycle (Pirke et al.). Pirke et al. concluded that even mild weight reduction diets can disrupt ovarian function in healthy, normal weight young females. They propose that impaired LH secretion is one of the mechanisms responsible (Pirke et al.).

Schweiger, Laessle, Schweiger, Herrman, Riedel, and Pirke (1988) conducted a longitudinal study in elite female athletes (n=18) and nonathletic controls (n=25) in an effort to link nutrition and stress with endocrine function. Blood was obtained by venipuncture daily (except on weekends) throughout one menstrual cycle. Subjects kept nutritional and training diaries and were instructed to maintain their usual training regimen. Four days were randomly selected from the nutritional diaries for analysis. At the end of the study, stress was evaluated by having the subjects rate their stress during the study period in 3 areas (work and studies; sport; partner, friends, and family) on a 5

point scale. These researchers found high correlations between caloric intake ($r = 0.70$, $p<.01$), and stress ($r = -.80$, $p<.01$) with luteal phase P secretion and suggest that low caloric intake and high stress are 2 factors contributing to the development of impaired luteal phase P secretion (Schweiger et al.). The methodology of energy intake in this study is problematic. The completeness and accuracy of energy intake records kept during an entire menstrual cycle should be questioned. Furthermore, the stress measures used do not appear to have been validated. Despite these methodological flaws, correlations were found between these variables and the athletes were shown to have significantly shorter luteal phases and significantly lower P and E_2 concentrations in the luteal phase than the control group. The exact mechanism for this HPO suppression remains elusive.

Many mechanisms for GnRH suppression in response to low metabolic fuel availability have been proposed. Schweiger (1991) suggests that since starvation and semistarvation have been shown to alter the function of neurotransmitters like norepinephrine or seratonin, and since those neurotransmitters are assumed to play a role in GnRH secretion, it follows that very low caloric intake may suppress the GnRH pulse generator (Schweiger, 1991). Bronson and Manning (1991) embellish this potential mechanism. They suggest that the GnRH pulse generator may react to insulin and that insulin levels could then influence the availability of amino acid substrates necessary for norepinephrine and seratonin synthesis (Bronson & Manning). Bronson and Manning suggest another possible mechanism to explain the relationship between the GnRH pulse generator and energy balance. They propose that low moment-to-moment availability of

the metabolic fuels glucose and fatty acids, may suppress GnRH pulses (Bronson & Manning).

In a recent study, Laughlin and Yen (1996), found a suppression of the GnRH/LH pulse generator in both amenorrheic and eumenorrheic athletes, however, this suppression was more marked in the amenorrheic group. These researchers concluded that this suppression appeared to be related to the reduced stimulatory effect of insulin-like growth factor I (IGF-I) in response to elevated insulin-like growth factor binding protein I (IGFBP-I) and to the central inhibition of GnRH by CRH (Laughlin & Yen). Jenkins and Grossman (1993) corroborate this finding suggesting that IGFBP-I may act on its own or with IGF-I as a peripheral signal indicating metabolic status and fuel reserves. Metabolic cues, therefore, may play an important role in GnRH suppression, however there are likely many other factors which contribute to or prevent reproductive hormone suppression.

The potential locations for the detection of metabolic cues, and the possible mechanisms by which these cues manifest the suppression of reproductive hormone secreting neurons in the brain have been examined. In their review, Wade et al. (1996) report that the best evidence suggests 3 possible locations where metabolic cues such as lipid and glucose could be detected: 1) forebrain GnRH-secreting or estrogen receptor immunoreactive (ERIR) containing neurons could detect metabolic fuel availability directly, 2) the metabolic cues could be detected in the periphery and transmitted to the forebrain, and 3) cues could be detected in the caudal hindbrain and transmitted to the forebrain. From these possible locations for metabolic fuel detection, Wade et al. (1996)

formulated a working hypothesis to explain where metabolic cues are detected and how they control reproductive hormone secretion by affecting GnRH-secreting and estrogen-binding effector neurons. They suggest that metabolic fuel availability is detected in the caudal hindbrain and the periphery. It is then transmitted to the forebrain, by a neural pathway and neurotransmitters, which control GnRH secretion (Wade et al.).

Body Fatness

Frisch (1987) has hypothesized that a critical body fat percentage (~22%) is necessary for the maintenance of female reproductive ability. Four mechanisms for this direct influence of body fat on reproductive status have been proposed: 1) since body fat converts androgens to estrogen by aromatization, body fat is a source of extragonadal estrogen, and a low body fat percentage would lower the amount of estrogen in circulation; 2) body fat influences the direction of estrogen metabolism; leaner women produce more catechol estrogens, the less potent form of this hormone; 3) obese females have a diminished capacity for estrogen to bind to sex-hormone binding globulin, which regulates the availability of estradiol to the brain and other target tissues; and 4) adipose tissue can store steroid hormones (Frisch). Frisch has also suggested that abnormal temperature regulation and changes in energy metabolism which accompany excessive leanness may indirectly influence reproductive function.

Snow, Barbieri, and Frisch (1989) prospectively studied 5 oarswomen who reported no menstrual cycle disturbances during the training year (group A), 5 oarswomen who reported periods of oligomenorrhea during intense training (group B), and a group of 4 sedentary females. The purpose of this investigation was to determine

the effect of exercise training on estrogen metabolism and to investigate the relationship between estrogen metabolism and training-associated menstrual dysfunction. In nonathletes, fatness was inversely associated with the extent of E_2 metabolized by 2-hydroxylase oxidation (Snow et al.). The extent of estradiol metabolized by 2-hydroxylase oxidation was measured in 2 out of the 3 training phases in 6 of the oarswomen, and in all training phases in 4 oarswomen. The group B oarswomen had increased 2-hydroxylase oxidation of estrogen across all training intensities (Snow et al.). The authors postulated that this increased estrogen oxidation was associated with the onset of menstrual disturbances during the intense training periods because during these periods, the oarswomen had very low body weight and body fat (Snow et al.). However, low body fatness cannot be the lone catalyst for menstrual cycle disturbances, since in this study, the group A athletes had lower body fatness than group B athletes during the most intense training period, yet experienced no menstrual disturbances despite similar net increases in estrogen oxidation (Snow et al.).

Although the critical body fat hypothesis was the only hypothesis which explained menstrual disturbances in athletes for some time, new hypotheses and growing evidence against the critical fat hypothesis has all but eliminated this theory from consideration. Estok, Rudy, and Just (1991) used 4 methods (body water and fat content, height to weight ratio, body weight, and weight loss) to determine body fat in a group of 94 female marathon runners with a 32% prevalence of menstrual irregularity as determined by responses to questions asked regarding the subjects' menstrual cycles. They found no clear relationship between menstrual irregularity and body fat (Estok et al.).

Sanborn, Albrecht, and Wagner (1987) also found convincing evidence against the critical fat hypothesis in their study of amenorrheic (n=7) and eumenorrheic (n=7) endurance runners. Height, weight, age, menarcheal age, weekly training mileage, days/week of training, years of training, maximum oxygen consumption, and body fat were not different between the amenorrheic and eumenorrheic runners (Sanborn et al.). These authors concluded that their data do not support the hypothesis that low body fatness in isolation causes menstrual disturbances (Sanborn et al.).

Exercise Training

Training volume and intensity, type of sport, age training started, and years of training all have been implicated as causes of menstrual disorders (Dale & Goldberg, 1982; Loucks & Horvath, 1985; Myburgh, Watkin, & Noakes, 1992). Many studies, both prospective and cross sectional, have attempted to reveal the link between training and menstrual disorders.

Prospective Studies

Bullen et al. (1985) conducted a prospective study to investigate the influence of exercise and weight loss on the induction of menstrual disorders in 28 untrained women. The 28 women were separated into a weight maintenance (WM) group (n=12) and a weight loss (WL) group (n=16). The chronological age of the weight maintenance and weight loss groups were 22 $\pm$0.5 years and 22 $\pm$ 0.8 years, respectively. The gynecologic ages of these groups were 10 $\pm$ 0.4 years and 10 $\pm$ 0.8 years, respectively. The subjects in this study were followed for 2 menstrual cycles. All women in this study had previous athletic experience but none were currently engaged in aerobic training.

The women ran twice a day for a mean distance of 7 miles per day at an intensity of 70-80% of their maximum oxygen consumption as determined from a treadmill test conducted before training was initiated. Overnight urine specimens were collected throughout the study. Urinary excretion of LH, FSH, estriol, free P and creatinine were measured. A biphasic basal body temperature curve, an ovulatory pattern of changes in gonadotropin and sex-steroid excretion, and a parabolic luteal phase P curve over a period of at least 9 days were indicators of a normal menstrual cycle. Records of menstrual cycle characteristics were also kept. The disturbances of reproductive function which occurred in this study were categorized as clinical disorders (abnormal bleeding and delayed menses) and hormonal disturbances (abnormal luteal function and loss of LH surge). The significant differences in menstrual cycle disturbances between the WM and WL groups occurred as delayed menses and loss of LH surge. One participant in the WM group had delayed menses whereas 12 subjects in the WL group had delayed menses. Five subjects in the WM group experienced a loss of LH surge in comparison to 13 subjects in the WL group. The researchers concluded from the hormonal measurements that exercise of sufficient intensity, particularly if coupled with weight loss, is capable of inducing reversible menstrual cycle disturbances (Bullen et al.).

Beitins et al. (1991) conducted a prospective study with identical protocol and subject groups to those used by Bullen et al. (1985). Of the 28 subjects in this study, 18 women had a total of 20 menstrual cycles with luteal phase defects during the exercise months. Luteal phase defects were classified as inadequate or shortened. Inadequate luteal phases were 9 days or longer but were characterized by decreased urinary free P

excretion. Shortened luteal phases were less than 9 days in duration. Of the 20 defective luteal phase cycles observed in this study, 6 were classified as inadequate and 14 were classified as short. Despite luteal phase shortening in 14 women, total cycle length was unchanged. In women with shortened luteal phases, the follicular phase lengthened. Women with inadequate luteal phases had normal luteal phase length. The incidence of luteal phase abnormalities was similar in both the WM and WL groups although in this study both groups experienced some weight loss with exercise (Beitins et al.). It is noteworthy that in the studies by Bullen et al. and by Beitins et al., the onset of exercise was sudden and weight loss occurred in both WL and WM groups. This leads to speculation that an energy imbalance may have been created by the sudden increase in energy expenditure created by the onset of exercise training, without adequate energy replacement, which led to weight loss in both subject groups.

A study by Bonen (1992) was conducted to determine whether recreational levels of training would induce shortened luteal phase menstrual cycles. Subjects were randomly assigned to 1 of 6 exercise groups for 2 or 4 months (1) <10 miles per week, 2 months running; 2) <10 miles per week, 4 months running; 3) 10-20 miles per week , 2 months running; 4) 10-20 miles per week, 4 months running; 5) 20-30 miles per week, 2 months running; 6) 20-30 miles per week, 4 months running) following 2 control menstrual cycles and 2 cycles where light calisthenics were performed. The 6 groups trained for either less than 10 miles per week, 10-20 miles per week, or 20-30 miles per week. All groups except 2 (<10 miles per week, 2 months running (n=12); 10-20 miles per week, 2 months running (n=13)) had 8 subjects. Eleven (11) of the subjects were

asked to perform 5 submaximal workloads on the treadmill while heart rate and VO_2 data were recorded. From regression equations, individual VO_2 estimates were derived from training heart rate data. The investigator estimated the training intensity during this study at 74% of VO_2 max. No changes in menstrual cycle function were observed in any of the training groups. Bonen explains this lack of significant findings as "attributable to the fact that weight losses were minimal, the women were gynecologically mature, and the training volume each week was below a critical threshold" (p.119). This research started with 132 subjects and concluded with 57 subjects. The researchers do not provide an explanation for this attrition rate. Furthermore, hormonal levels of LH, FSH, and P in this study were determined from single blood samples taken on days 1 or 2, 6 or 7, 9 or 10, 12, 13, 14, 15, 16, 17, 18, 20 or 21, 23 or 24, etc. during every second menstrual cycle. Due to the pulsatile nature of hormone secretion, and the individual variations which occur in women, this sampling method would not give an accurate hormonal profile. Bullen et al. (1985) used an overnight urine collection to determine hormonal levels. Although this method does not show individual pulses, it gives an indication of total hormone levels during each night of the study. Bonen may not have found significant menstrual cycle impairment because the training intensity in this study was not strictly monitored. The exercise adherence is also questionable since no significant changes in body weight, fatness, or VO_2 max occurred despite 2-4 months of running up to 30 miles per week. Energy intake data, which could possibly explain the lack of body weight and fat changes, were not collected. In addition, the initial training status of the subjects in this study is poorly defined. Subjects were excluded from the study if they

were "physically very active" but precise volume, intensity, and VO_2 max guidelines were not reported.

Rogol et al. (1992) prospectively examined the effect of exercise intensity on physiological and endocrine responses. Twenty-three women (gynecologic age 17.8 $\pm$ 0.9 years, chronological age 18-40 years) were randomly assigned to 1 of 3 groups. One group (n=9) trained at an intensity equal to their lactate thresholds (LT), one group (n=8) trained at an intensity above their lactate thresholds and one group (n=6) served as a control group and did not train. The women trained 4-6 times per week. Volume, not frequency was prescribed. The women were not participating in regular physical activity (the equivalent of 10 miles of running per week) prior to the start of the study. Menstrual cycle phase lengths and pulsatile LH release were evaluated at 4 month intervals during the one-year study. Single daily blood samples obtained from day 9 until the onset of bleeding were taken at baseline, and every fourth month during the study. These daily samples were analyzed for E_2, P, LH and FSH. In addition, on the day 4 or 5 of every fourth menstrual cycle, blood samples were taken every 10 minutes for 28 hours and analyzed for E_2, P, LH and FSH. The group training at LT trained at a velocity equivalent to the velocity that they were at when LT was attained during a VO_2 max test. The authors did not report where the training occurred (treadmill vs. track). The group which trained above LT ran 1-3 days per week at LT and 3 days per week between LT and their VO_2 max. A slight decrease in the menstrual cycle length of the group training at lactate threshold was observed (P=.10) from the first to the last observation. Luteal phase length was significantly decreased from the first to the last observation in the group

training above lactate threshold (P=.04). These researchers deemed the observed changes in menstrual status as not significant. This conclusion is controversial since others (Prior, 1990; Prior & Vigna, 1991; Loucks, 1990) have found that shortened luteal phases are common in athletes and may have detrimental effects on bone density due to the trophic effect of progesterone on bone (Prior, 1990). The training in the Rogol et al. study was unsupervised for 1-3 sessions per week. The adherence to the training protocol in these unsupervised sessions is questionable since very little change in body fatness or weight occurred in these previously untrained women, despite similar pre-and post-training caloric intake. Some important hormonal changes which may have occurred in response to the training may have been missed due to the long intervals (4 months) between testing sessions. Also, as with Bonen (1992), reporting the cycle and phase length as group mean values may have camouflaged important individual data. Finally, the women in the Rogol et al. study were of advanced gynecologic age when compared to the women in the prospective study by Bullen et al. (1985). This gynecologic maturity may have a preventive influence on the development of menstrual disorders.

Cross Sectional Studies

A cross sectional study by Lund Hetland, Haarbo, and Christiansen (1993) investigated the prevalence of exercise-related menstrual and sex hormone disturbances. Two hundred and five (205) premenopausal women whose training ranged from normally active (mean weekly running distance = 20 km per week, n = 88, mean age = 34.5 years), to recreational runners (mean weekly running distance = 48 km per week, n = 89, mean age = 34.0 years), to elite runners (mean weekly running distance 67 km per

week, n = 28, mean age = 29.1 years) were recruited. The age that training started and the years of prior training were not reported. Serum E_2, P, LH and FSH were measured on a random day of the menstrual cycle and again 10 and 20 days later. The runners were classified by menstrual status based on questionnaire responses as regular (interval between 24 and 33 days), amenorrheic (no bleeding for at least 3 months during the last 12), or oligomenorrheic (one or more cycles delayed more than 30 days in the past 12 months). On all measures, there was a progression from the normally active members, to the recreational runners, to the elite runners. The results indicated that sex hormone fluctuations in the menstrual cycle were vulnerable to physical activity. The levels of E_2, P, and LH were all shown to be lower in elite runners (average VO_2 max 60.2 mL/min/kg) when compared to normally active (VO_2 max 45 mL/min/kg) women. Menstrual disturbances were found to increase with the amount and intensity of running (Lund Hetland et al.).

Cumming, Vickovic, Wall, and Fluker (1985) studied 6 runners (aged 20-30 years) who ran a minimum of 32 km per week and 4 sedentary controls (aged 23-32 years). All subjects were eumenorrheic. LH was measured every 15 minutes for 6 hours in the early follicular phase of the menstrual cycle. All of the control subjects had normal LH serum levels and pulsatile patterns. In the runners, LH pulse frequency, amplitude and area under the LH curve were diminished when compared to the sedentary control group (Cumming et al.). Cumming et al. suggested that exercise-associated amenorrhea may be a further development of the changes in the GnRH-pituitary function exhibited in this study by eumenorrheic runners.

Cumming, Vickovik, Wall, Fluker, and Belcastro (1985) conducted further studies to determine whether acute exercise had any effect on pulsatile LH release. These researchers studied 6 eumenorrheic runners, training a minimum of 32 km per week, during 2 menstrual cycles. In the early follicular phase (days 1-6) of the first cycle, blood samples were obtained every 15 minutes for 6 hours. During the early follicular phase of the second cycle, blood samples were obtained every 15 minutes for 30 minutes before, 60 minutes during, and 6 hours following a 60-minute treadmill run at 11.2 km per hour with a slope calculated to be equivalent to 60% of VO_2 max. The LH pulse frequency was reduced after exercise when compared to the values obtained at rest. Pulse amplitude and area under the 6-hour LH curve were not significantly different between testing times. Cumming et al. concluded that acute exercise has an inhibitory effect on LH pulse frequency in eumenorrheic runners.

Summary of Exercise Training Studies

Despite these studies, it is not well understood how these training factors influence the menstrual cycle. There seems to be a consensus that intense training decreases LH, progesterone and estradiol in some women. In eumenorrheic women, diminished LH pulsatility has been observed in the early follicular phase (Cumming, Vickovic, Wall, & Fluker, 1985). This diminished LH pulsatility may be a precursor to exercise-associated amenorrhea (Cumming et al.). Luteal phase abnormalities which include shortened and insufficient luteal phases, are also common in elite female athletes (Loucks, 1990). Luteal suppression is conventionally considered to be an intermediate stage which progresses with severe training to amenorrhea (Loucks). GnRH pulse

generator suppression is the probable cause of these abnormalities. The mechanism for this GnRH suppression is unknown, yet alterations in neurotransmitters may be a potential cause.

Insufficient energy intake, low body fatness, and intense training are all challenges to the body's energy demands which have been implicated as predisposing factors to menstrual disorders. A decrease in body fatness is likely the outcome of insufficient energy replacement coupled with high energy output necessitated by intense exercise, rather than a causal mechanism for menstrual disorders. Furthermore, body fat stores do not necessarily indicate adequate fatty acid availability (Wade et al., 1996). Wade et al. report that the best available evidence indicates that as long as the energy expended in physical activity is replaced, menstrual disorders need not occur.

Stress

The HPA is activated by both physical and psychological stress. Both CRH and GnRH are hypothalamic hormones. CRH may suppress GnRH secretion from the arcuate neurons of the hypothalamus (Stratakis & Chrousos, 1995). The pathophysiological mechanism of menstrual disorders has not been fully elucidated. However, there is evidence that it involves the hypoactivation of the HPO and hyperactivation of the HPA (Ferin, 1993; Tolis & Diamanti, 1995). Hyperactivation of the HPA is often quantified by measuring cortisol concentrations peripherally (De Souza et al., 1991; Ding et al., 1988; Loucks et al., 1989). Many types of stress have been shown to affect cortisol secretion. Physical stressors include hypoglycemia, trauma, and heavy exercise (Rhoades & Pflanzer, 1992). Psychological stressors include acute anxiety and chronic anxiety

(Rhoades & Pflanzer). Elite athletic females have been shown to have higher cortisol levels than their sedentary peers (Loucks et al.). These high cortisol levels may represent the physical stress of training, the emotional and physical stress of competition, and the chronic emotional stress of the athletic subculture (Lopiano & Zotos, 1992).

Psychological Stress

Psychological stress has long been implicated as a cause of menstrual disorders. In 1944, Whitacre and Barrera described menstrual disturbances in 50 % of the female internees in a World War II internment camp. They report the cessation of menses abruptly after bombing or soon after internment, before a food deficiency could have an effect (Whitacre & Barrera). These authors postulated that severe psychic shock, worry and suppression of ovarian function by the autonomic nervous system were the cause of the menstrual disorders in their subjects (Whitacre & Barrera). This study demonstrates an extreme of psychological stress.

Facchinetti, Demyttenaere, Fioroni, Neri, and Genazzani (1992) emphasize the importance of exposing stressful life events and diagnosing psychopathology in the evaluation of menstrual disorders. Athletic menstrual disorders may also be associated with affective disorders. In a study of 13 amenorrheic (AR) and 19 eumenorrheic runners (ER), 8 AR were diagnosed with eating disorders compared to 0 ER (Gadpaille, Sanborn, & Wagner, 1987). In addition, 3 AR were diagnosed with bipolar disorder or major depression versus 0 ER (Gadpaille et al.). The age, age at menarche, miles of training per week, height and body fatness were similar in both groups (Gadpaille et al.). Depression in non-athletic populations has also been associated with hypercortisolism (Gold, Loriaux

et al., 1986; Kaye, Gwirtsman, George, Ebert, Jimerson, Tomai, Chrousos, & Gold, 1987).

Another affective disorder, anorexia nervosa (AN) affects many athletic women (Leon, 1991). One of the diagnostic criteria for AN is amenorrhea (DSM-IV, 1994). This 100% incidence of amenorrhea in AN patients suggests a relationship between amenorrhea, weight loss, and nutritional state (Tolis & Diamanti, 1995). Yet, this generalization is confounded by the fact that 1 out of 5 AN patients are amenorrheic prior to weight loss (Tolis & Diamanti). AN is also associated with hypercortisolism (Gold, Gwirtsman et al., 1986; Hotta et al., 1986; Kaye et al., 1987). AN patients who have regained weight show a concurrent drop in CRH (Gold, Gwirtsman et al; Kaye et al.). AN studies have shown that the pituitary responsiveness to CRH is appropriate in the hormonal milieu of hypercortisolism, suggesting a defect at or above the hypothalamus (Gold, Gwirtsman et al.).

In addition to severe psychic shock and chronic affective disorders, acute stressors may also cause reproductive hormone suppression. Cameron et al. (1993) described a series of studies they performed on male rhesus monkeys to determine whether or not the psychological stress associated with fasting suppresses pulsatile LH secretion. The monkeys were overfed on the day before fasting in order to maintain the monkeys in a metabolically fed state on the day of fasting. Blood was sampled every 20 minutes for 12 hours on the day following overfeeding. The overfeeding was successful in preventing the suppression of LH pulse frequency normally found after fasting. However, the behavioural agitation associated with fasting was not prevented. The authors argue that

the lack of reproductive hormone suppression coupled with the agitated behaviour provide strong evidence against the hypothesis that reproductive hormone suppression is caused by the stress of missing a meal (Cameron et al., 1993).

Physical Stress

Physical stress and its effects on the HPO is often studied by pharmacologically catalyzing the stress response with CRH injections (Calogero et al., 1988; Hotta et al., 1986). In the rat, Petraglia, Sutton, Vale, and Plotsky (1987) demonstrated that central injection of CRH attenuated LH pulsatility by a central mechanism to inhibit GnRH secretion into the hypophysial portal circulation. These researchers also found that the CRH suppression of GnRH was enhanced by endogenous opioids (Petraglia et al.).

Cameron et al. (1993) found that male rhesus monkey cortisol and ACTH levels were higher in the fasted state; they found no correlations between the magnitude of the fasting-induced rise in cortisol and the degree of fasting-induced LH suppression (Cameron et al.). These authors went on to examine the effect of exogenous hydrocortisone acetate (HCA) on LH secretion. They found that the exogenous HCA did not suppress LH secretion (Cameron et al.). Finally these authors administered dexamethasone to fasting monkeys and found that the dexamethasone prevented the fasting associated rise in cortisol, but did not prevent the fasting induced suppression of LH (Cameron et al.).

The physical stress of exercise has also been associated with menstrual disorders. CRH secretion is regulated by glucocorticoid feedback (Ferin et al., 1993). Cortisol inhibits the HPO at all levels (Stratakis & Chrousos, 1995). Elevated cortisol levels have

been reported in both amenorrheic and eumenorrheic athletes (De Souza et al., 1994). De Souza et al. (1991) studied adrenal response to exercise in 16 female runners 18-37 years old. The women in this study were classified as amenorrheic (n=8) and eumenorrheic (n=8). The amenorrheic athletes in this study had significantly higher baseline serum cortisol levels ($P<0.05$) than their eumenorrheic counterparts. The cortisol response to both maximal and submaximal exercise was blunted by more than 50% in the amenorrheic runners as determined by blood samples taken 4, 20, and 40 minutes during the recovery period from exercise. The blunted cortisol response seemed to be due to reduced adrenal sensitivity in this study. The authors give 2 possible explanations for the reduced adrenal sensitivity: 1) negative feedback of cortisol, or 2) the adrenal gland may be secreting at near maximum capacity even under resting conditions (De Souza et al., 1991). It is evident that cortisol, a measure of both emotional and physiological stress, may be an important mediator of menstrual function.

Other investigators have studied hypercortisolism in patients with hypothalamic amenorrhea (HA). Biller et al. (1990) found that women with HA (n=10) had higher cortisol levels than eumenorrheic subjects (n=20) (determined from blood samples obtained every 10 minutes for 24-hours and 24-hour urinary free cortisol), and blunted cortisol responsiveness to exogenous CRH administration (determined from serum cortisol samples taken 15 minutes before and 15, 30, 60, 90, and 120 minutes after a bolus injection of ovine CRH). Berga et al. (1989) studied LH pulsatility and pituitary responses in women with HA (n=15) and in eumenorrheic women (n=16) during their early follicular phase to administration of GnRH and CRH. In this study, blood samples

were obtained every 15 minutes for 24 hours. These researchers found LH pulse frequency diminished in the HA group and that there was similar pituitary responsiveness to bolus CRH and GnRH injections in both subject groups (Berga et al.). These data suggest that the decreased LH pulsatility in HA women may be mediated by supra hypothalamic factors. In the rat, Rivier and Vale (1984) found that CRH lowered plasma LH levels. This effect was present in gonadectomized and adrenalectomized rats and is therefore not a steroid mediated effect (Rivier & Vale). These findings suggest defects in the central neuroregulation of the secretion of pituitary hormones.

While fasting, exercise, and affective disorders may be associated with a rise in cortisol levels, hypercortisolism may not be the sole causative mechanism for menstrual disorders in athletes. Physical and psychological stress may exacerbate HPO suppression but likely are not the sole causative factors. Hyperactivation of the HPA may be a side effect of metabolic substrate insufficiency rather than a direct cause of menstrual disorders.

Menstrual History

In their review of athletic amenorrhea, Loucks and Horvath (1985) give epidemiological evidence that reproductive maturity may protect against menstrual disorders. The incidence of amenorrhea is higher in younger women (Loucks & Horvath). Parity may also protect against menstrual disorders since women who have borne children have a lower incidence of amenorrhea (Loucks & Horvath). Age at menarche may also affect the development of menstrual disorders in athletic females. However, because investigators do not often report whether training was initiated before

or after menarche, comparisons are difficult (Loucks & Horvath). No causal mechanisms for the protective aspect of reproductive maturity were suggested.

Other

Endogenous Opiates

CRH also inhibits GnRH secretion indirectly via beta-endorphin. Beta-endorphin, an endogenous opioid peptide, inhibits GnRH release (Stratakis & Chrousos, 1995), and is a modulator of GnRH pulse frequency (Ferin et al., 1993). CRH secretion stimulates the conversion of proopiomelanocortin (POMC) to ACTH and beta-endorphin (Ferin et al.). Beta endorphin release is stimulated by progesterone. When present, progesterone is directly responsible for a decrease in LH pulse frequency (Ferin et al.). Periods of low endogenous opioid peptide activity in the hypothalamus and low progesterone reflect periods of high GnRH pulse frequency; whereas periods of high endogenous peptide activity and high progesterone reflect low GnRH pulse frequency (Couzinet & Schaison, 1993; Ferin et al.).

Gindoff and Ferin (1987) demonstrated that CRH suppression of gonadotropin release from the anterior pituitary in the primate is modulated by endogenous opioid peptides. This conclusion was made since naloxone, an opioid receptor antagonist, prevented the suppression of gonadotropin release that was present in ovariectomized rhesus monkeys which were given a continuous 5-hour CRH infusion (Gindoff & Ferin).

However, Cameron et al. (1993) reported that the LH supression seen in fasting rhesus monkeys could not be reversed with an 8-hour infusion of naloxone. Although fasting is an energetic stress, and stress has been shown to activate endogenous opioid

secretion, their results indicated that endogenous opioid systems do not appear to play a central role in LH supression caused by fasting (Cameron et al.)

In eumenorrheic women, naloxone has been shown to cause increases in LH levels in the luteal but not the follicular phase (Genazzani & Petraglia, 1989; Genazzani, Genazzani, Volpogni, Pianazzi, Li, Surico, & Petraglia, 1993; Genazzani, Petraglia, De Ramundo, Genazzani, Amato, Algeri, Galassi, Botticelli, & Bidzinska, 1991). This effect is likely a function of the steroidal milieu around the hypothalamus at the time of naloxone administration (Genazzani & Petraglia, 1989; Jenkins & Grossman, 1993).

Quigley et al. (1980) found that a 4-hour naloxone infusion increased LH levels in 4 of 8 amenorrheic women. Wildt & Leyendecker (1987) gave 3 amenorrheic women oral naltrexone (a long acting opiod receptor antagonist) for 28 days and reported that all 3 women had ovulated during the 28 day administration. In a double blind study with oral naltrexone and a placebo, Remorgida et al. (1990) reported that 18 of 24 amenorrheic women had ovulatory cycles with naltrexone while 8 of 24 had ovulatory cycles with the placebo. However, these findings contradicted those reported by Cumming and Rebar (1983) and Samuels et al. (1991), who reported that an infusion of naloxone did not change LH levels in athletic amenorrheic women. This evidence suggests that amenorrhea may be of heterogenous origin and while some cases may involve opioid induced GnRH suppression other cases may have other causal mechanisms. Differences in acute naloxone administration versus the chronic administration of the longer acting naltrexone may also confound the comparison of these studies.

Neurotransmitters

The suppression of GnRH by CRH is likely a result of a neurotransmitter receptor-mediated process (Ulrich, Nowara, & Rossmanith, 1994). Noradrenaline and gamma-amino-butyric acid (GABA) may stimulate GnRH release, while catechol-estrogens (De Souza & Metzger, 1991) and dopamine (Quigley et al., 1980) may inhibit GnRH release.

Noradrenaline

In rhesus monkeys, infusions of alpha-adrenergic receptor antagonists rapidly extinguish spontaneous GnRH pulses (Velduis, 1990). Studies of the precise role of adrenergic neurons in the control of the GnRH pulse generator in humans have been inconclusive (Veldhuis, 1990).

GABA

Corticotropin releasing hormone has an inhibitory effect on GnRH secretion. Stimulation of GABA receptors has been shown to indirectly increase GnRH secretion by suppressing CRH secretion (Judd et al., 1995). Judd et al. conducted a study on the effect Alprazolam, a GABA receptor agonist, on LH pulsatility. This study had 3 subject groups: 2 groups of normally menstruating women (one in the early follicular phase (EFP) (n=6), one group in the mid luteal phase (MLP) (n=6), and one group of women with stress-related anovulation (SRA) (n=6)). Blood samples were collected every 10 minutes for 10 hours. The SRA group showed an increase in LH pulse amplitude, frequency, and mean serum LH after Alprazolam administration (Judd et al.). In the EFP women, Alprazolam increased the LH pulse amplitude and decreased the pulse frequency.

In the MLP women, Alprazolam had no significant effect on pulse amplitude or frequency (Judd et al.). The authors of this study hypothesize that stimulation of the GABA receptors inhibits CRH release which has an inhibitory effect of GnRH pulsatility (Judd et al.). This effect is much more pronounced in the SRA women than in the normally menstruating women because in the normally menstruating women, the CRH inhibition of GnRH is minimal (Judd et al.). In the EFP women, the reduced LH pulse frequency may be a result of GABA inhibition of noradrenergic neurons which are a stimulus for GnRH pulsatility (Judd et al.). In the MLP women, the authors postulate that the high P levels in this phase of the cycle correspond with an increase in opioid levels which inhibit the noradrenergic neurons (Judd et al.).

Calogero et al. (1988) studied GABA receptor agonists and their effect on CRH secretion in the rat.. Their results show that stimulation of GABA receptors have a suppressive effect on CRH secretion. These authors postulate that this CRH suppression is a result of lowered seratonin levels (Calogero et al.). However, Ulrich, et al. (1994) demonstrated through the use of a seratonin antagonist (ondansetron) that the blockade of this neurotransmitter failed to change LH secretion. Therefore, the effect of GABA on CRH and GnRH secretion may or may not be mediated by seratonin.

Contradicting results were also found by Jarry, Leonhardt, and Wuttke (1993), who studied the GnRH pulse generator and the neuronal interactions that affect it in the rat. These researchers inhibited GnRH release by either electrical lesions or by infusion of GABA (Jarry et al.). These conflicting results on the effect of GABA on GnRH secretion may be explained by the fact that the previous studies stimulated endogenous

GABA secretion pharmacologically and found a stimulatory effect of GABA on GnRH secretion while the study by Jarry et al. infused exogenous GABA which may have suppressed GnRH secretion via negative feedback from high GABA concentrations.

Dopamine

Quigley et al. (1980) conducted a study using metoclopramide, a dopamine receptor antagonist, to investigate the relationship of dopamine on LH pulsatility in normally menstruating women (n=9) in the EFP and in women with hypothalamic amenorrhea (n=8) (HA). Blood samples were obtained every 15 minutes for 1 hour before and 3 hours after metoclopramide injection. The results demonstrated that metoclopramide had no effect on the LH pulsatility of normally menstruating women (Quigley et al.). In contrast, 4 of 8 women with HA showed significantly increased LH levels after metoclopramide infusion (Quigley et al.). The authors suggest that the 4 non-responders may have had a more severe form of HA or may have HA of a different origin (Quigley et al.).

Catechol Estrogens

Russell et al. (1984b) studied 13 female competitive swimmers over 2 training years. During periods of high volume training, oligomenorrhea was observed in 5 of the 13 athletes (Russell et al., 1984b). The oligomenorrhea was associated with significantly lower levels of LH and higher levels of catechol estrogens and beta-endorphins when compared to more moderate training periods (Russell et al., 1984b). These hormonal levels were determined in the athletes from 2 blood samples taken on a training day 30 minutes before swimming. The first blood sample was taken during a strenuous training

period and the second one year later during a moderate training period. No effort was made to control the day of the menstrual cycle that blood was taken from the swimmers. Despite the methodological flaws in this study, the authors suggest that elevated levels of catechol estrogens and beta-endorphin may be potential causal mechanisms for oligomenorrhea (Russell et al., 1984b).

Undetermined Factors

It is possible that menstrual disorders in female athletes are caused by a mechanism which has not been revealed. Until the exact causal mechanism for these disorders is revealed, it remains possible that research in this area has missed the potential cause completely.

Part VI-Summary of the Menstrual Disordered Athlete Profile

Athletes with and without menstrual disorders have been shown to have varying hormonal profiles. It is significant that even eumenorrheic athletes have been shown to have suppressed HPOs. In general, athletes exhibit decreased LH pulse frequency in the early follicular phase, shortened or inadequate luteal phases, and hypercortisolism. Gynecologically mature athletes with an established menstrual cycle appear to be less vulnerable to menstrual disorders.

There are many conflicting findings reported in the literature with respect to causal factors for menstrual disorders. There are potentially many factors which cause menstrual disorders. The primary locus of altered menstrual function in athletes is likely the GnRH secreting neurons of the arcuate nucleus. Energetic challenges and high stress are the main predisposing factors which have been implicated as predisposing factors to

menstrual disorders. Low body fatness has also been implicated as a cause of menstrual disorders. However, low body fatness is likely a consequence of increased energy expenditure in exercise and insufficient energy replacement rather than a direct cause of menstrual disorders. It is also possible that there are other, undetermined factors which predispose athletes to develop menstrual disorders.

Energetic challenges may include low energy availability, high energy expenditure and insufficient energy replacement. Very small changes in energy availability can elicit rapid suppression of LH pulses. Changes in glucose, lipid, and amino acid availability may affect neurotransmitter synthesis. Neurotransmitters have been shown to play a role in GnRH secretion. Rapid onset of training and periods of high volume training are energy drains which have also been associated with menstrual disorders. Insufficient or delayed energy replacement rather than exercise itself is likely to predispose individuals to menstrual disorders.

Physical and psychological stress both activate the HPA which has been shown to suppress the HPO. Individuals with low LH levels seem particularly vulnerable to high levels of CRH and endogenous opioids.

The causal mechanisms which link predisposing factors to menstrual disorders are unclear. The hypothesis suggested by Wade et al. (1996) seems to be the most plausible explanation for athletic menstrual disorders at this time. This hypothesis involves the detection of metabolic cues which are then transmitted to the GnRH secreting neurons where action may be taken to suppress reproductive physiology and behaviours in times of low energy availability (Wade et al., 1996).

Part VII-Health Consequences of Menstrual Disorders

There are several clinical consequences of menstrual disorders. These are: 1) skeletal problems, 2) infertility, and 3) increased risk of coronary heart disease (CHD). Estrogen has a protective effect on the skeleton and in the prevention of CHD (Constantini, 1994).

The most deleterious consequence of exercise-associated menstrual dysfunction is likely its influence on bone density. Premature bone loss is a major health concern for amenorrheic athletes. Trabecular bone has a higher turnover rate than cortical bone and is therefore more susceptible to bone loss (Drinkwater, 1992). Low concentrations of circulating estrogens are thought to be the main cause of premature bone loss in amenorrheic athletes (Drinkwater, 1992). Progesterone may have a trophic effect on bone (Prior, 1990). Thus, the low progesterone levels seen in amenorrheic athletes and in athletes with shortened or insufficient luteal phases, may prevent this action. High cortisol levels may have a catabolic effect on bone and may potentially contribute to decreased bone density (Constantini, 1994). Decreased bone mineral density in amenorrheic female athletes has been reported (Carbon, 1992; Drinkwater et al., 1984; Drinkwater, 1992; Fruth & Worrell, 1995). The long term effects of the bone density loss that occurs in amenorrheic women are unknown. Preliminary studies in women with anorexia nervosa have shown that with weight gain and with the resumption of menses, some lost bone density may be restored (Iketani et al., 1995). However, females who have experienced prolonged bouts of amenorrhea may not achieve the bone mineral density of their eumenorrheic peers (Drinkwater, 1992; Iketani, et al.,1995). There is

strong evidence which links normal ovarian function with the acquisition and maintenance of bone mass, and low estrogen levels with a reduction in bone mass (Snow-Harter, 1994). Shortened and insufficient luteal phase cycles and decreased progesterone levels may also contribute to bone loss (Prior & Vigna, 1991; Prior, Vigna, & McKay, 1992).

A woman must ovulate in order to become pregnant. If reproductive hormone levels are not sufficient to support ovulation, a woman is infertile. Exercise associated infertility is usually reversible with a reduction of training or weight gain (Constantini, 1994; Prior & Vigna, 1985).

Exercise is associated with elevated high-density lipoproteins (HDL) and decreased low-density lipoproteins (LDL). Estradiol also enhances lipolysis in muscle and adipose tissue, and inhibits gluconeogenesis and glycogenolysis (Constantini, 1994). Conversely, insufficient estradiol concentrations may negatively affect plasma lipids and accelerate the development of atherosclerosis (Constantini, 1994). Lamon-Fava , Fisher, Nelson, Evans, Millar, Ordovas, and Schaefer (1989) studied the effects of exercise and menstrual status on plasma lipids and apolipoproteins. Their study consisted of 3 groups: amenorrheic runners, eumenorrheic runners, and sedentary controls. The results showed that the apolipoprotein A-I (apo A-I) levels, the major protein of HDL, were not different between groups (Lamon-Fava et al.). The plasma apolipoprotein B (apo B) levels, the major protein of LDL, were lower in both athletic groups than in the control women (Lamon-Fava et al.). However, amenorrheic women had lower serum estradiol levels, apo A-I levels, and apo A-I/apo B ratios than eumenorrheic runners (Lamon-Fava et al.).

Lower estradiol levels in the amenorrheic group are significant since estrogens increase plasma apo A-I levels (Lamon-Fava et al.). These researchers concluded that the benefits of exercise on plasma lipoproteins may be negated in amenorrheic runners (Lamon-Fava et al.). Friday, Drinkwater, Bruemmer, Chesnut and Chait (1993) similarly concluded that high levels of plasma LDL and cholesterol may adversely affect cardiovascular risk. However, Friday et al. found a concurrent rise in HDL in the amenorrheic athletes which they suggest may neutralize the risk of cardiovascular disease in amenorrheic athletes.

Part VIII-Treatment of Menstrual Disorders

Since the cause of menstrual disorders is elusive, it is difficult to treat the problem. It is possible, however, to attempt to prevent the detrimental effects of hypoestrogen secretion on bone. Oral contraceptive pills are often prescribed for this purpose (Marshall, 1994). Oral contraceptives create predictable cycles, decreased risk of breast or endometrial cancer and less dysmenorrhea in addition to preventing excessive bone loss (Prior & Vigna, 1985). Unfortunately, oral contraceptives are not without side effects. There is a minimal risk of thrombosis, a variable tendency to increase body fatness, and a possible lowering of maximum oxygen consumption associated with oral contraceptives that may discourage elite female athletes (Prior & Vigna, 1985).

Part IX-Future Directions

The high incidence of menstrual disorders in athletes suggests the need for further research in this area. Controlled studies with appropriate and accurate measures need to be done so that this problem is better understood. Menstrual disorders are multi-faceted disorders which likely have many inter-related causative factors. Individuals involved in

all areas of sport, from the athlete to the coach, to the administrator, to the sport science and sport medicine support teams must be educated about the detrimental effects of menstrual disorders. It is only with understanding that preventive models can be constructed.

Part X- Summary

Based on previous research, there are some predisposing factors associated with menstrual disorders which may may be relatively more influential to menstrual function than others. Energetic challenges resulting from high energy output and/or low energy intake together with an activated HPA may be the main factors which contribute to menstrual disorders. Low body fat is likely a consequence of a negative energy balance rather than a main causal mechanism for menstrual disorders. Body composition, stress, training, and energy intake have not been previously examined simultaneously and prospectively in relation to menstrual cycle integrity. However, many previous studies have examined some of these relationships in isolation. Typically, the athletes in these studies have been runners aged 20-30 years. Other sports and other age groups have not been thoroughly investigated. Previous prospective studies have implemented training programs but have not followed the athletes in their usual training environment.

Chapter Three
Methodology

Study Design

The relationships among 24-hour urinary cortisol, energy intake, body composition, and training on the menstrual cycles of elite female synchronized swimmers were investigated in a 10-month prospective study. The following variables were investigated in 9 elite female synchronized swimmers and 8 sedentary females: energy intake, questionnaires (Derogatis Stress Profile, Ways of Coping, frequency and duration of menstrual cycles, Pictorial Blood Loss Assessment Chart), body fatness, maximum oxygen consumption (VO_2max), LH pulsatility, luteal phase progesterone, and 24-hour urinary cortisol. In addition to having a control group and an experimental group, this study also had a single subject design where each subject served as her own control so that individual changes could be monitored throughout the 10 month investigation period.

Testing times were chosen to correspond to the athletes' preparatory phase (November), pre-competitive phase (December), early competitive season (February), and competitive season (March-April).

Ethical Considerations

This study was approved by the Ethics Committee in the Faculty of Physical Education and Recreation, by the Cooperative Activities Program of the Faculty of Education, and also by Research Ethics Board of the Faculty of Medicine.

Before participating in the study, all aspects of the study were explained to the subjects and then informed consent was sought (Appendix A). Parental or guardian consent was obtained for any individuals who were under 18 years of age (Appendix B).

The subjects were told that they could withdraw from the study at any time without penalty.

There was minimal risk associated with participation in this study. It was possible that discomfort and/or bruising could result from insertion of the indwelling catheter. There was also a small risk of infection at the site of the venipuncture. This risk was minimized by using sterile technique and universal precautions. The total volume of blood collected was 100 mL, an amount less than a blood donation which should present no danger to healthy volunteers.

There was also a small risk associated with the maximum oxygen uptake test (VO_2 max). During this maximal effort test, subjects could experience abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases, heart rhythm disturbances or heart attack. These effects are very rare in healthy populations. Subjects were observed very closely during and after this test, and the test was terminated if signs of distress were observed. Subjects were also informed that they could terminate any testing procedures at any time. Despite these potential risks, no adverse effects associated with participation in this study were reported.

Inclusion/Exclusion Criteria and Screening

The inclusion criteria for this study were: 1) age 15 -21 years, 2) female, 3) chronically trained (synchro group). The exclusion criteria for this study were: 1) ages below 15, 2) use of hormonal medications such as estrogen, progesterone, cortisone, growth hormone, anabolic steroids, or thyroid hormones in the past 6 months, 3) parental refusal (for subjects under 18 years of age), and 4) smoking. An additional set of

exclusion criteria for the control group were: 1) regular physical activity (see definitions in Chapter 1), 2) a $V0_2$ max exceeding 39 mL/kg/min, 3) oligomenorrhea or amenorrhea, and 4) abnormal eating restrictions. Subjects were asked to perform a VO_2 max test and to answer food frequency (Appendix C), menstrual history/medication (Appendix D), and training history (Appendix E) (synchro group) questionnaires to determine whether or not they met the above criteria. Once accepted into the study, the control group was asked to maintain their current lifestyle throughout the study - specifically to avoid regular physical activity, and smoking. Both groups were asked to avoid the use of oral contraceptives or other hormonal medication. If the subjects requested information on alternative birth control options, they were put in contact with an appropriate source of information.

Sampling/Recruitment

A sample of 9 elite female synchronized swimmers were asked to participate in this study. This sample size was chosen to represent the typical number of swimmers on a synchronized swimming team (8 plus 1 alternate). Synchronized swimming is a team sport where a training volume and intensity is generally consistent between team members. Having the entire experimental group from the same club of synchronized swimmers controlled for many differences in training and increased the internal validity of this study.

The swimmers were asked to participate based on their order of finish at the 1996 National Championships (May 6-14) and on the rankings of the club tryouts held in September, 1996. Recruitment started with the top 10 swimmers and broadened as

necessary based on subject willingness and acceptance into the study. Six out of 11 swimmers from the senior team and 3 swimmers from 2 15-17 age group teams (one swimmer from a team of 8, 2 swimmers from another team of 7) were selected. All swimmers were chronically trained (see definitions in Chapter 1). The swimmers were initially contacted by telephone by the investigator, which was followed by an information meeting where a letter (Appendix F), study information (Appendix G), and a schematic overview of the study (Figure 3.1) were given to the subjects.

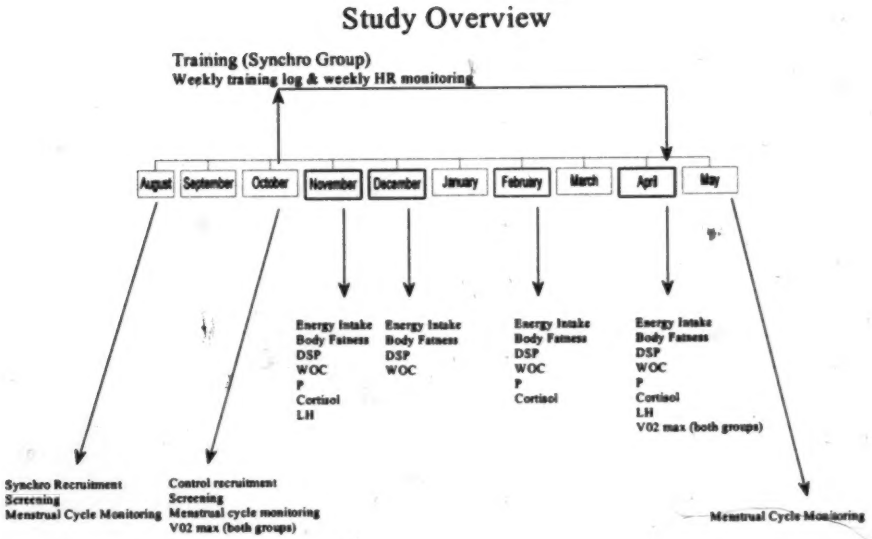
The control group (n=9) was matched by both age and gynecologic age to the experimental group. Metcalf et al. (1983) reported that unfailing ovulation occurred in 71.8% of women 5-8 years from menarche. Therefore, a control group was necessary to separate the effects of training from those of natural maturation of the reproductive axis. The control group was also sedentary to control for the effect of physical activity on the menstrual cycle.

The control group was difficult to recruit due to the exclusion criteria, invasive nature and duration of this study. Some of the control group, therefore, was recruited later than the synchro group. Two members of the control group were recruited in October (including the one subject who later dropped out of the study in February), 3 in November, and 4 in January. The subjects that were recruited later had a more condensed testing schedule, however each member of the control group completed all aspects of the study (see revised overview for the late recruits in Appendix H).

The control group consisted of sedentary student volunteers self-selected from high-school and post-secondary schools. At each school, the control group was selected

on the basis of obtained permission and convenience. Two members of the control group were recruited in a large Health Education class by giving a brief overview of the study on an overhead projector and then asking interested volunteers to approach the researcher. One of these volunteers gave the study information to a friend who was later selected into the study. Three control group subjects were given the study information by members of the senior team and one of these subjects brought a friend. Another control group subject was a coach of a synchronized swimming team known to the researcher and the final subject was selected randomly from the birthdate roster at a local high school. The subjects in the control group were approached personally and given the study information and the schematic overview of the study.

A total sample of 17 subjects completed this study. There were 9 subjects in the experimental group and 9 in the control group. However, one of the control group subjects dropped out of the study in February due to the time commitment involved and illnesses in her family. Each individual received their personal results once the study was completed.



Key: VO_2 max = maximum oxygen consumption, DSP = Derogatis Stress Profile, WOC = Ways of Coping Questionnaire, P = progesterone, LH = luteinizing hormone

Figure 3.1 Schematic overview of the study

Measures and Methods

The following measures were taken according to the testing schedule outlined in Figure 3.2.

I) Body Composition

Height: Height was measured with a set square and measuring tape to the nearest 0.2 cm. Subjects were barefoot and they stood with their feet together and their posterior side against a wall.

Weight: Weight was measured to the nearest 0.5 kg on a beam balance scale with the subject wearing shorts and a t-shirt. The scale was calibrated with a set of known weights before each testing day.

Skinfolds: Five skinfolds (triceps, biceps, subscapular, suprailiac, and medial calf) were measured according to the Canadian Standardized Test of Fitness (CSTF) (CSTF Operations Manual, 1986) protocols with Harpenden calipers to the nearest 0.2 mm. A sum of 5 skinfolds was calculated and compared to Canadian population norms for age and gender (CSTF Operations Manual, 1986).

II) Energy Intake

A 3-day energy record (see sample page and instruction sheet given to subjects in Appendix I) was used to assess daily nutrient intake. Energy intake was recorded on 2 weekdays and one weekend day. The Food Processor VI program (ESHA Research, Salem, Oregon) was used to quantify daily grams of protein, carbohydrate, fat intake, total energy intake, and the percentage that each macronutrient (protein, carbohydrate, and fat) contributed to the total daily energy intake. Mean 3-day intakes (grams and percentages of total energy intake) of each macronutrient were calculated.

III) Training

Athletes were asked to keep a daily training log. In this log they recorded the volume, type, and duration of training each day (Appendix J). The coach was also asked to submit her daily training plans at the end of each week. The researcher then combined

Independent Variable	Measuring Tools	Testing Times									
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Body Composition	1) 5-skinfold thichnesses				X	X		X		X	
	2) Height				X	X		X		X	
	3) Weight				X	X		X		X	
Stress	4) 24-hour urinary cortisol				X			X		X	
	5) WOC				X	X		X		X	
	6) DSP				X	X		X		X	
Training	7) Weekly athlete training log			X	X	X	X	X	X	X	
	8) Weekly coach training log			X	X	X	X	X	X	X	
	9) Weekly heart rate monitoring			X	X	X	X	X	X	X	
	10) VO_2 max			X						X	
Energy Intake	11) 3-day energy intake				X	X		X		X	

Dependent Variable	Measuring Tools	Testing Times									
		Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
Menstrual Cycle Integrity	1) LH pulsatility				X					X	
	2) Luteal phase salivary P				X			X		X	
	3) Menstrual cycle frequency/ duration log	X	X	X	X	X	X	X	X	X	X
	4) PBAC			X	X	X	X	X	X	X	X

Key: WOC = Ways of Coping Questionnaire, DSP = Derogatis Stress Profile, VO_2 max = maximum oxygen consumption, LH = luteinizing hormone, P = progesterone, PBAC = Pictorial Blood Loss Assessment Chart

Figure 3.2 Testing schedule

this information and documented the number of training hours per day, the number of weight training sessions per week, and the number of training days per week for each athlete.

Training intensity was measured on a standardized day each week by having the athletes wear Polar® heart rate monitors throughout their afternoon practice. Due to a limited number of heart rate monitors (n = 3), the heart rate monitors were given to different athletes each week. Therefore, each athlete wore a heart rate monitor once every third week. In total each athlete wore the heart rate monitor 6-7 times during the study. The heart rate data from the practices were downloaded and analyzed for the average, high, and low heart rates over the training sessions.

IV) Hormones

Cortisol: Since exercise has been associated with increased cortisol levels (De Souza et al., 1991), cortisol was measured in the experimental group on a non-training day, a minimum of 21 hours after the last bout of exercise (typically urine was collected on a Sunday and Saturday training was finished at 11:00 a.m.). In the control group, cortisol was measured on a convenient weekend day. Originally, urine was to be collected on the first non-training day after the onset of menstruation. This standardization proved to be an inconvenience for the subjects. Since there is no evidence that concentrations of cortisol vary across the menstrual cycle (Kanaley, Boileau, Bahr, Misner, & Nelson, 1992; Stewart et al., 1993), urine collection occurred during the specified testing months on convenient days (non-training days for the experimental group). It should be noted that creatinine was not measured to indicate

incomplete urine collections, therefore the accuracy of this data is unknown. The importance of not missing collection times was emphasized to each subject.

The subjects were provided with a 4 L jug and were instructed to begin urine collection after emptying their bladders in the morning, and to continue for a full 24 hours (Herring, Mole, Meredith, & Stern, 1992) (see Appendix K for instructions given to subjects). The collection was completed after the collection of the first void the following morning (Herring et al., 1992). The urine was picked up by the researcher and transported to the lab immediately following the 24-hour collection. The total 24-hour volume was then measured. Two 100 mL well mixed aliquots were saved and frozen at -80 °C until analyzed. Once the samples were thawed, a urine extraction procedure was followed before radio-mmunoassay (RIA) analysis for extracted urinary free cortisol (UFC) was performed using an INCSTAR ^{125}I RIA Kit (procedures for urine extraction and UFC RIA were obtained from the INCSTAR Clinical Assays™ GammaCoat™ Cortisol ^{125}I RIA Kit Instruction Manual). After analysis, extracted UFC was calculated as described in the INCSTAR Clinical Assays™ CammaCoat® Cortisol ^{125}I RIA Kit Instruction Manual with the following difference. According to the INCSTAR manual, extraction efficiency is recommended for each sample. This process is used to determine the efficiency of methylene chloride extraction and is reported to be 99±14% (INCSTAR manual). Using one extraction tube for each sample tube would double the cost of this RIA procedure, therefore only one extraction efficiency tube was used for every third sample tube. This gave an indication of methylene chloride extraction proficiency and kept RIA costs to a minimum. However, UFC is calculated using the difference between

efficiency tube concentration and the sample tube concentration. Because extraction efficiency was unable to be assessed for every tube, in this study UFC was calculated using the expected difference between the efficiency tube concentration and the sample tube concentration (see appendix L for the extracted UFC formula and sample calculations).

Luteinizing Hormone: In the experimental group, blood sampling occurred a minimum of 12 hours after the last exercise bout since LH levels are affected by acute exercise (Hall-Jurkowski, 1982). The subjects reported to the Women's Health and Physical Activity Lab at 7:45 a.m. during the early follicular phase of their menstrual cycles (day 1-7). Oligomenorrheic and amenorrheic subjects were tested on an arbitrary day. An indwelling intravenous (IV) catheter, connected to a 3 way stopcock and a 1 L bag of saline, was inserted into a forearm or antecubital vein by a physician or qualified nurse. Subjects were then instructed to sit for 30 minutes with the saline running. Beginning at 8:30 a.m., a 2 cc. blood sample was taken every 10 minutes for 8 hours using 2 syringes connected to a 3-way stopcock. The intravenous catheter was removed at 4:30 p.m.. During the sampling, subjects were allowed to consume typical meals and beverages and ambulate as necessary. Excessive activity and sleeping were not permitted. Blood samples were immediately transferred to glass test tubes, allowed to clot for 45 minutes followed by centrifugation with the serum frozen at -80°C until analyzed using an INCSTAR ^{125}I RIA Kit following the procedures described in the Clinical Assays™ GammaDab® LH ^{125}I RIA Kit Instruction Manual.

LH pulse analysis was conducted via cluster analysis using the Munro 1.1 (Zaristow software, Scotland) hormone pulse profile program. The specific settings for the LH pulse analysis were determined using the method described by Veldhuis and Johnson (1986) which allows the researcher flexibility in selecting cluster sizes of test peaks and pre- and post-peak nadirs while minimizing the potential for false-positive errors. In this study, a cluster configuration of 2 points for the nadir and 2 points for the peak was selected. These values have also been used in other LH pulsatility research (Berga et al., 1989; Judd et al., 1995; Suh et al., 1988). A *t* statistic of 2.0 was used in this study and has been used in other studies (Judd et al., 1995) to provide optimal sensitivity (>90%) and accuracy.

Progesterone: Progesterone was measured in the saliva of the subjects. This method has been shown to be highly correlated to serum progesterone measures with the advantage of being non-invasive (Vuorento et al., 1989). Beginning on day 12 of their menstrual cycle and continuing until the subsequent period, each subject was asked to collect saliva into a Salivette® (Sarstedt Inc.) (consisting of a plastic test tube and a cotton pellet) each morning prior to eating or drinking or brushing her teeth. The subjects were instructed to store the tubes in their home freezer until they collected all of the samples necessary for that testing session, at which time the containers were delivered to the lab or retrieved by the researcher (see Appendix M for the saliva collection instructions given to each subject). For the amenorrheic and oligomenorrheic athletes, an arbitrary starting day was selected and samples were collected for 24 days. Samples were stored at -80°C until analyzed. At 7:00 p.m. on the night before the saliva tubes

were to be analyzed, they were removed from the freezer and placed in a 4°C refrigerator to thaw. The following morning, to extract the saliva from the cotton pellet, the tubes were centrifuged at 3,000 G for 10 minutes. Analysis of progesterone was conducted using the INCSTAR ^{125}I RIA Kit. Procedures used were obtained from the Clinical Assays™ GammaCoat™ Progesterone ^{125}I RIA Kit Instruction Manual with the following exception. In order to increase the sensitivity of the standard curve at low concentrations, the known standards were diluted to produce the following concentrations: 0, 0.15, 0.3, 0.5, and 1.0 ng/mL. The known concentration quality control serum was also diluted to concentrations of 0.5 and 1.0 ng/mL. In addition, the incubation time was increased from the recommended 60-70 minutes to 90 minutes which allowed for improved binding performance. The mean progesterone concentration, and area under the progesterone curve were used to indicate luteal phase sufficiency.

Hormone Analysis: All hormones were assayed using INCSTAR Corporation (Stillwater, Minnesota, U.S.A.) commercially prepared ^{125}I RIA Kits. To minimize inter-assay variability, all hormonal samples from the same subject were analyzed in duplicate on the same testing day. Serum controls and the number of computer iterations required to plot the standard curve for the assays were used to assess the validity of the assays. The precision of the assay was measured by a computer generated coefficient of variability (CV). Acceptable CV's are 6-15% (Chard, 1987).

V) Questionnaires

Questionnaires administered were: 1) menstrual history, 2) menstrual frequency and duration, 3) Pictoral Blood Loss Assessment Chart (PBAC), 4) food frequency, 5)

training history (experimental group), 6) Derogatis Stress Profile (DSP), and 7) Ways of Coping (WOC).

Menstrual History: The menstrual history questionnaire (Appendix D) indicated the gynecologic age of the subjects and demonstrated past and present menstrual patterns.

Menstrual Frequency and Duration: Menstrual frequency and duration were recorded monthly on a specified form (Appendix N).

PBAC: The PBAC forms were completed each month (Appendix O). Scores from this chart have been found by Higham et al. (1990) to be highly correlated ($r = 0.74$) to menstrual blood loss.

Food Fequency Questionnaire: The food frequency questionnaire (Appendix C) was used to screen for a normal mixed diet. This type of questionnaire provides a qualitative measure of a subject's usual intake over a given time period (Freudenheim, 1993).

Training History Questionnaire: The training history questionnaire (Appendix E) was used to identify the athletes' prior training.

DSP: The DSP (Appendix P) was designed to measure stress (Derogatis, 1995). The DSP is a 77 item self-report inventory (Derogatis, 1987). The DSP was derived from the interactional theory of stress and has 3 principal domains: environmental events, personality mediators, and emotional responses (Derogatis, 1987). The DSP provides scores for 11 dimensions of stress (time pressure, driven behaviour, attitude posture, relaxation potential, role definition, vocational environment, domestic environment, health posture, hostility, anxiety, depression) which each represent one of the 3 domains

(Derogatis, 1987). Raw scores should be used for group comparisons whereas comparisons of individual data to normative data should use T-scores (Derogatis, 1995). From the scores of the DSP, the researcher is able to calculate total stress scores and subjective stress scores for each subject (Derogatis, 1987). Highly stressed individuals should have high DSP scores. Derogatis (1995) reports that the DSP is valid and reliable as a screening or outcome measure of stress.

WOC: The WOC (Appendix Q) developed by Folkman & Lazarus (1988), categorizes the way that an individual has coped with a stressful life event in the past week. The Ways of Coping Questionnaire (WOC) is a 66-item questionnaire which uses a 4-point Likert scale (Folkman & Lazarus, 1988). There are 8 sub-scales on this questionnaire: 1) confrontation coping, 2) distancing, 3) self-controlling, 4) seeking social support, 5) accepting responsibility, 6) escape-avoidance, 7) planful problem solving, and 8) positive reappraisal. Raw scores for each scale describe coping effort for each coping type, whereas relative scores describe the proportion of effort represented by each type of coping (Folkman & Lazarus, 1988). Preliminary work has shown that this questionnaire is a reliable measure of coping and has both face and construct validity (Folkman & Lazarus, 1988). The WOC has been used in longitudinal studies (Folkman, 1997).

VI) Maximum Oxygen Consumption (VO_2 max)

Subjects were instructed to report to the lab wearing comfortable clothing and to have eaten a small low fat, low fibre meal at least 2 hours prior. Subjects were also instructed to avoid vigorous exercise for 24 hours prior to the test. At the lab, subjects

warmed up on a Monark cycle ergometer for 5-10 minutes. Once the warm up was completed, the subjects were connected to a Sensormedic Horizon Metabolic Cart by way of mouthpiece and hose. The metabolic cart collected and analyzed expired respiratory gases continuously. Average expired respiratory gas concentrations were calculated and printed every 15 seconds. Heart rate was monitored continously via a Polar® heart rate monitor and recorded every minute during the test. Subjects started the graded exercise test at a resistance of 0.5 kp and at a pedal speed of 60 rpm. The resistance was increased by 0.5 kp every 2 minutes and subjects were instructed to maintain the pedal speed throughout the test. The test was continued until volitional exhaustion. VO_2 max was considered to be obtained if 2 of the following criteria were met: 1) volitional exhaustion, 2) a RER $\geq$ 1.1, 3) a maximum heart rate within 10 % of the age predicted maximum (220-age), 4) a peak and plateau in VO_2 despite increases in workload.

Data Analysis

To determine differences in physical characteristics between group means at the pre-test, independent T-tests were calculated. To determine the differences between testing times within the same group and between groups for each variable, data were also analyzed using an analysis of variance (ANOVA) with repeated measures. Where significance was obtained, multiple T-tests were conducted to determine where the differences existed. An alpha level was set *a priori* at 0.05, and where multiple T-tests were used, the Bonferroni correction was applied.

Further, data were analyzed using a Pearson's Product correlation matrix to determine where statistically significant relationships existed between the variables. Due

to the large number of variables in this study, representative measures were chosen which best described each independent variable for correlation purposes. Since each variable was tested a different number of times, a decision was made to examine the correlations between chosen representative variables for pre- and post-test results only in the following manner: the average of pre and post test results, the difference between pre- and post-test results, and pre- and post-test results for each variable independently.

In addition to the group data analysis, individual data were also considered. To demonstrate individual changes during the study, graphs for each hormonal variable were drawn for each individual and tables were presented for all other data. Case studies were selected for presentation based on athletic performance and outlying data.

Chapter Four
Results

Subject Characteristics

Subject characteristics at test 1 are shown in Table 4.1. The synchronized swimmers (SS) were significantly leaner than the control group (CG), as determined by t-tests for independent sample means on body mass index (BMI), the sum of 5 skinfold thicknesses (SOS), and Canadian Standardized Test of Fitness (CSTF) percentile ranking for skinfold thicknesses. There were no statistically significant differences between the groups for age, gynecologic age, height, and weight at the start of the study.

Table 4.1 Subject Characteristics (mean ± SE)

Group	Age (yrs)	Gyn. Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	SOS (mm)	CSTF %ile
Synchro n = 9							
Mean	17.2	3.4	170.4	60.9	20.9[a]	66.4[a]	43.9[a]
SE	0.5	0.6	2.3	2.7	0.6	5.0	6.9
Control n = 8							
Mean	17.9	5.0	163.8	63.5	23.6	95.0	14.4
SE	0.6	0.7	2.5	3.7	1.2	5.0	3.2

a = significant difference between group means at $p < 0.05$

Training

The pre- and post-study maximum oxygen consumption (VO_2 max) test results for both the SS and the CG are shown in Table 4.2. The repeated measures ANOVA conducted on the absolute and relative VO_2 max results revealed a significant main effect of group, $F(1,15)=9.73$, $p<.05$ and $F(1,15)=28.78$, $p<.05$, respectively. The SS had higher maximum oxygen consumption than the CG. No main effects of time and no interaction effects were observed in the absolute or relative VO_2 max results.

The SS were chronically trained. As of May 1, 1997, the mean number of years of synchro training for this group was 7.2 (SE ± 1.1; range 3-12). All SS trained 6 days per week for 10.6 (SE ± 0.2; range 10-11) months of the year. During each training week the SS had 7-8 training sessions. The SS and their coaches submitted 30 weeks of training logs. The mean number of training hours per week was calculated for each athlete. The mean number of training hours per week for all athletes during the 8 documented training months of the study was 17.2 hours (SE ± 0.73, range 14.6-19.7). Table 4.3 shows the mean number of training hours and the mean training heart rate (HR) for all athletes during each month of the study. These training hours include water time, 3 weight training sessions per week (1 hour per session), 1-2 dryland sessions per week (approximately 1 hour per session; dryland includes running, cycling, flexibility exercises and isolated work on synchro-specific muscle groups), and 1-2 landrill sessions per week (approximately 30 minutes per session; landrill reinforces the routine choreography on land).

Heart rates were recorded for each athlete an average of 6.7 (SE ± 0.2; range 5-7) times during the study. Heart rates for each individual were recorded by a Polar® HR monitor every 15 seconds during a 2-3 hour practice and an average HR for the entire training session was calculated. Table 4.4 shows mean HR for each athlete during the study, the maximum HR for each athlete, and the percentage of the maximum HR that the mean training HR for each athlete represents. The maximum HR for each athlete was the highest HR recorded during the VO_2 max tests.

Table 4.2 Maximum Oxygen Consumption (VO_2 max): Pre- and Post-Test (mean ±SE)

Group	$VO_{2\,max}$ Pre-test (l/min)	$VO_{2\,max}$ Post-test (l/min)	$VO_{2\,max}$ Pre-test (ml/kg/min)	$VO_{2\,max}$ Post-test (ml/kg/min)
SS n =9	**2.69[a]**	**2.69**	**44.02[a]**	**45.11**
SE	0.17	0.16	1.61	1.40
Range	2.07-3.71	2.29-3.90	37.50-49.40	40.30-51.60
CG n = 9	**1.99**	**2.22**	**31.65**	**35.34**
SE	0.11	0.07	1.71	2.00
Range	1.56-2.45	1.85-2.44	24.80-38.70	26.20-42.50

a = significant main effect of group at $p < 0.05$

Table 4.3 Training Hours and Training Heart Rates (HR) During each Month of the Study (mean ± SE)

Month of Study	Hours/Week	SE Range	Mean HR (bpm)	SE Range
October	**12.7**	1.3/9.4-16.5	**106.0**	3.5/100.0-112.0
November	**16.4**	0.8/14.0-18.1	**120.8**	2.6/116.8-125.5
December	**11.4**	3.1/0.3-17.0	**117.5**	6.2/111.3-123.7
January	**17.0**	0.9/15.0-18.7	**115.3**	2.7/109.0-120.7
February	**19.0**	1.2/16.8-21.3	**115.8**	1.4/113.7-118.3
March	**16.3**	3.1/6.0-21.5	**112.2**	3.0/103.3-116.7
April	**17.4**	3.4/12.4-24.0	**109.7**	3.3/103.3-114.3

Table 4.4 Heart Rates (HR) for each Athlete from all HR Recording Sessions and the Percentage of each Athlete's Maximum HR (Max HR) that the HR Represents (mean ± SE)

ID #	Mean HR (bpm)	SE Range	Max HR (bpm)	% of Max HR
101	**120.4**	2.4/112-127	**187**	**64**
102	**121.1**	2.9/111-134	**198**	**61**
103	**110.7**	3.0/99-121	**190**	**58**
104	**118.2**	3.6/109-133	**194**	**61**
105	**107.7**	3.5/92-117	**194**	**56**
106	**125.6**	2.8/113-135	**192**	**65**
107	**107.7**	4.2/92-128	**193**	**56**
108	**109.3**	2.8/100-119	**184**	**59**
109	**107.6**	3.4/96-115	**196**	**54**

Anthropometry

Mean height, weight, BMI, SOS, and CSTF percentile ranking results for all testing times are shown in Table 4.5. A repeated measures ANOVA was conducted for all anthropometric variables. Height results showed a significant main effect of group, $F(1,15)=5.60$, $p<.05$; however there was no main effect of time and no interaction effect. The SS were statistically significantly taller than the CG subjects. Weight results showed no main effect of group and no interaction effect yet a main effect of time was demonstrated, $F(3,45)=3.15$, $p<.05$. Multiple paired t-tests using the Bonferroni correction revealed no significant differences for all subjects between testing times. Main effects of group and time were shown in the BMI ANOVA results, $F(1,15)=6.32$, $p<.05$ and $F(3,45)=3.67$, $p<.05$ respectively. The SS had statistically significantly lower BMIs than the CG. Multiple paired t-tests using the Bonferroni correction revealed no significant differences for all subjects between testing times. There was no significant interaction effect in the BMI results. The SOS results showed significant group and time main effects, and an interaction effect $F(1,15)=29.3$, $p<.05$; $F(3,45)=8.02$, $p<.05$; and $F(3,45)=6.77$, $p<.05$ respectively. Multiple paired t-tests using the Bonferroni correction showed that the SS decreased their mean SOS from test 1 to test 2, from test 1 to test 3, and from test 1 to test 4. Figure 4.1 demonstrates that the SS decreased their mean SOS from test 1 to test 2 and then maintained this body fat loss for the duration of the study. The SS were statistically significantly leaner than the CG at all testing times. The CSTF percentile data also showed significant group, time and interaction main effects, $F(1,15)=39.18$, $p<.05$; $F(3,45)=11.70$, $p<.05$; and $F(3,45)=12.23$, $p<.05$, respectively.

Multiple paired t-tests using the Bonferroni correction showed significant differences in the SS from test 1 to test 2, from test 1 to test 3, and from test 1 to test 4. As demonstrated in Figure 4.1, the SS decreased their body fat (and therefore their CSTF percentile) from test 1 to test 2 and then maintained this loss throughout the study. Again, the SS were leaner than the CG at all testing times.

Table 4.5 Anthropometric Measures for Both Groups at each Testing Session (mean ± SE)

Measure	Test 1		Test 2		Test 3		Test 4	
	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8
Height (cm)	**170.4[a]**	**163.8**	**170.2**	**162.2**	**170.6**	**162.2**	**170.4**	**162.4**
SE	2.3	2.4	2.3	2.6	2.1	2.6	2.0	2.7
Range	163.0-184.6	154.0-171.5	162.1-184.7	154.3-172	163.1-183.6	154.0-172.5	163.1-183.3	154.0-172.1
Weight (kg)	**60.9[b]**	**63.5**	**58.9**	**62.0**	**59.7**	**61.4**	**59.4**	**61.7**
SE	2.7	3.7	2.7	3.3	2.7	3.4	2.4	3.1
Range	48.7-78.0	54.4-83.7	46.9-76.9	55.2-83.9	48.4-78.1	53.3-83.6	49.2-75.4	54.7-80.8
BMI (kg/m^2)	**20.9[a,b]**	**23.6**	**20.3**	**23.6**	**20.4**	**23.4**	**20.4**	**23.4**
SE	0.6	1.2	0.6	1.1	0.5	1.2	0.5	1.0
Range	18.2-23.0	19.4-29.6	17.3-22.5	19.9-30.1	17.7-23.2	18.9-30.0	17.9-22.4	19.6-28.6
SOS (mm)	**66.4[c]**	**94.1**	**56.7**	**95.4**	**58.8**	**92.5**	**55.2**	**92.7**
SE	5.0	5.5	3.4	6.1	3.2	5.7	2.4	5.3
Range	50.0-99.4	76.2-114.7	43.3-78.7	80.1-117.6	45.9-80.3	77.2-114.6	48.5-69.6	77.1-111.3
CSTF % ile	**43.9[c]**	**14.4**	**59.4**	**13.8**	**55.6**	**16.3**	**62.8**	**14.4**
SE	6.9	3.2	5.9	2.6	5.7	3.1	4.6	2.9
Range	10.0-75.0	5.0-25.0	25.0-85.0	5.0-20.0	20.0-80.0	5.0-25.0	35.0-75.0	5.0-25.0

a = significant main effect of group at $p < .05$
b = significant main effect of time at $p < .05$
c = significant main interaction effect at $p < .05$

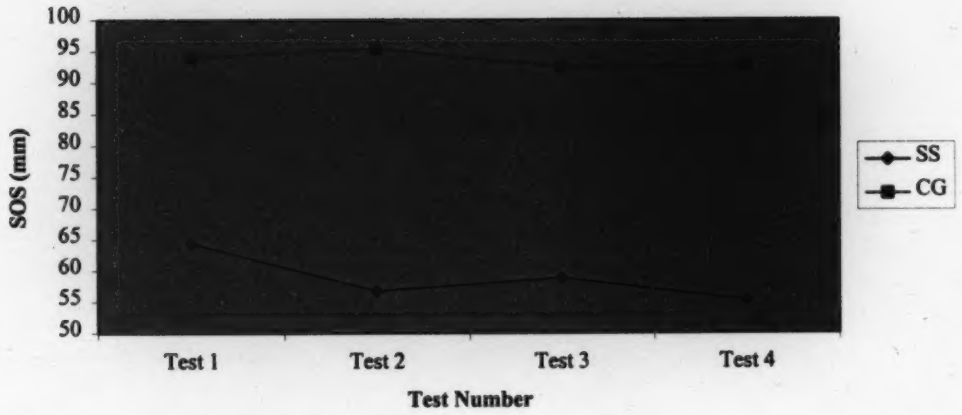


Figure 4.1 SOS (mean)

Energy Intake

Mean 3-day energy intakes were calculated for each subject at each testing time. Group means were calculated and are shown in Table 4.6. One subject in the control group only recorded her energy intake for one of the 3 days on the third 3-day record. Her results were therefore excluded from the ANOVA analysis. The repeated measures ANOVA analysis conducted demonstrated no significant differences between groups or between testing times for any nutrient with the exception of carbohydrate grams consumed, where a main effect of time was observed $F(3,42)=2.89$, $p<.05$ (Figure 4.2). Multiple paired t-tests using the Bonferroni correction revealed no significant differences for all subjects between testing times.

Table 4.6 3-Day Energy Intakes (mean ± SE)

Energy	Test 1		Test 2		Test 3		Test 4	
	SS n = 9	CG n = 7	SS n = 9	CG n = 7	SS n = 9	CG n = 7	SS n = 9	CG n = 7
Pro (g)	**78.1**	**73.8**	**70.8**	**69.1**	**65.4**	**68.0**	**73.2**	**64.1**
SE	7.7	5.2	8.6	7.8	6.2	7.0	6.2	6.1
Range	39.7-115.9	53.7-96.7	25.2-104.4	44.6-95.2	30.1-88.6	40.3-86.3	45.7-103.2	40.6-97.8
Pro (%)	**13.4**	**15.0**	**14.7**	**15.7**	**13.9**	**13.9**	**14.6**	**12.7**
SE	0.8	0.5	1.0	1.3	0.9	0.8	1.3	0.9
Range	9.0-17.3	12.7-17.3	9.7-18.3	9.3-20.0	9.3-17.7	11.0-17.0	9.7-20.7	9.7-17.3
CHO (g)	**366.1[b]**	**292.6**	**272.9**	**262.6**	**284.9**	**278.9**	**307.2**	**288.1**
SE	22.5	10.8	18.5	34.3	26.8	31.8	30.2	11.4
Range	246.5-446.2	254.9-339.6	176.4-333.4	150.5-443.7	158.3-429.5	139.3-393.3	191.5-509.1	235.6-321.7
CHO (%)	**64.3**	**58.1**	**59.0**	**57.7**	**61.4**	**58.0**	**58.8**	**58.5**
SE	3.1	3.1	3.8	1.8	2.9	3.7	3.1	2.0
Range	54.0-81.7	46.0-69.7	48.0-78.3	49.0-64.3	46.7-73.7	43.7-70.0	42.0-68.7	50.3-65.7
Fat (g)	**57.7**	**61.9**	**56.4**	**52.7**	**52.5**	**64.5**	**60.7**	**65.8**
SE	8.8	8.8	7.9	8.9	5.6	11.8	5.6	6.8
Range	19.9-94.9	34.1-101.0	12.8-87.8	20.6-98.1	16.7-75.8	20.9-103.8	38.9-84.8	36.5-97.1
Fat (%)	**21.9**	**26.9**	**25.8**	**26.0**	**24.6**	**28.2**	**26.5**	**28.6**
SE	2.5	3.0	3.0	2.4	2.2	3.3	2.1	2.0
Range	10.0-30.0	17.3-41.3	11.3-37.7	18.0-36.7	14.0-35.3	19.0-42.0	18.0-37.3	20.7-37.0
Calories	**2242.8**	**1999.7**	**1854.1**	**1789.3**	**1892.8**	**1947.2**	**1989.6**	**1976.0**
SE	147.6	97.5	147.7	225.2	166.3	202.1	117.8	116.4
Range	1613.1-2939.4	1567.5-2443.3	973.4-2433.3	970.3-2972.6	1059.6-2697.9	896.0-2434.5	1527.2-2660.0	1439.7-2362.7

b = significant main effect of time at $p<.05$

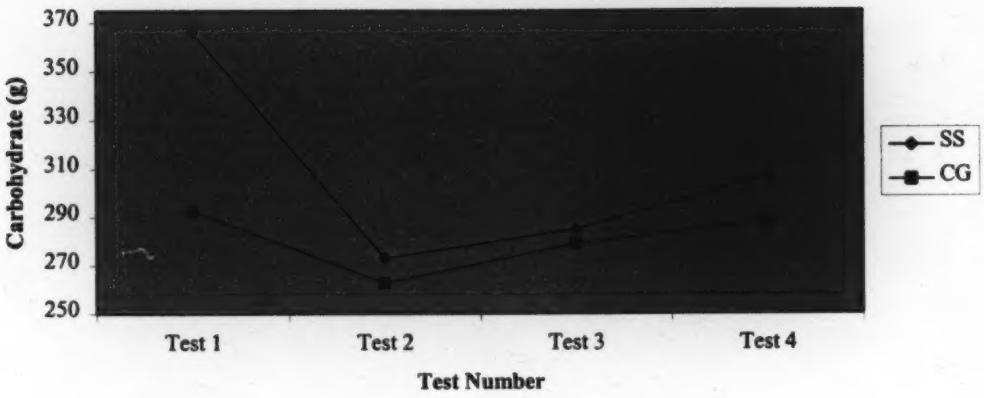


Figure 4.2 Carbohydrate (mean grams) consumed

Stress

Derogatis Stress Profile (DSP)

The mean Derogatis Stress Profile (DSP) domain scores for each group are shown in Table 4.7. At all 4 testing times, n = 1-3 of the SS did not rate their subjective stress. These subjects were omitted when the ANOVA was conducted on the subjective stress score (SSS) in the SS group. The repeated measures ANOVA revealed no significant differences between groups or testing times for the domain scores. However, when individual subscales were examined, 2 differences were noted. Attitude posture and health posture showed significant main effects of group $F(1,15)=4.43$, $p<.05$ and $F(1,15)=8.59$, $p<.05$ respectively, with the SS scoring higher than the CG on attitude posture and the CG scoring higher than the SS on health posture. Attitude posture is a subscale in the personality mediators domain and health posture is a component of the environmental events domain.

Ways of Coping Questionnaire (WOC)

Ways of Coping Questionnaire (WOC) mean relative scores for all subscales are shown in Table 4.8. No significant main effects or interaction effects were observed at any of the testing times when a repeated measures ANOVA was conducted.

Urinary Free Cortisol (UFC)

Mean urinary free cortisol (UFC) concentrations for each group at each testing session are shown in Table 4.9. A repeated measures ANOVA revealed no significant main effects or interaction effects. Wide individual variability was observed in the UFC results. Figures 4.3 and 4.4 demonstrate this individual variability in UFC over the 3 testing times in the SS and in the CG, respectively.

Table 4.7 Derogatis Stress Profile Domain Scores, Total Stress Scores and Subjective Stress Scores (mean ± SE)

Domain	Test 1		Test 2		Test 3		Test 4	
	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8
Personality Mediators	**237**	**216**	**234**	**213**	**240**	**214**	**235**	**202**
SE	9	8	12	7	13	8	12	9
Range	195-285	181-253	180-289	184-239	185-296	177-241	198-290	159-229
Environmental Events	**147**	**146**	**143**	**143**	**140**	**147**	**141**	**141**
SE	6	5	7	6	6	6	9	9
Range	127-175	121-165	121-179	109-166	118-168	115-165	106-183	91-172
Emotional Response	**152**	**136**	**149**	**138**	**153**	**137**	**154**	**132**
SE	10	7	10	6	9	5	13	8
Range	116-212	105-175	114-202	112-159	118-198	115-159	92-218	98-174
Subjective Stress Score	**53 (n = 6)**	**46 (n = 8)**	**59 (n = 6)**	**48 (n = 8)**	**50 (n = 6)**	**55 (n = 8)**	**54 (n = 6)**	**47 (n = 8)**
SE	5	8	5	6	8	7	7	8
Range	27-62	7-63	33-77	19-69	24-79	32-75	27-89	13-82
Total Stress Score	**537**	**498**	**526**	**494**	**534**	**498**	**530**	**474**
SE	23	15	24	14	26	13	30	20
Range	441-669	440-554	415-656	425-526	428-662	455-555	419-677	377-553

Table 4.8 Ways of Coping Relative Subscale Scores (mean ± SE)

Subscale (Relative Scores)	Test 1		Test 2		Test 3		Test 4	
	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8
Confrontive Coping	**0.07**	**0.09**	**0.08**	**0.09**	**0.08**	**0.10**	**0.12**	**0.09**
SE	0.01	0.01	0.02	0.01	0.02	0.01	0.02	0.01
Range	0.02-0.14	0.04-0.13	0.02-0.19	0.04-0.14	0.03-0.20	0.05-0.16	0.03-0.25	0.05-0.15
Distancing	**0.13**	**0.10**	**0.12**	**0.12**	**0.13**	**0.10**	**0.12**	**0.13**
SE	0.02	0.02	0.02	0.01	0.01	0.01	0.03	0.01
Range	0.03-0.23	0.04-0.18	0.06-0.22	0.04-0.16	0.05-0.18	0.03-0.13	0.02-0.24	0.09-0.17
Self-Controlling	**0.16**	**0.15**	**0.15**	**0.16**	**0.13**	**0.15**	**0.15**	**0.13**
SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Range	0.11-0.25	0.11-0.22	0.09-0.20	0.12-0.20	0.04-0.16	0.10-0.17	0.12-0.19	0.08-0.17
Seeking Social Support	**0.15**	**0.18**	**0.12**	**0.16**	**0.12**	**0.16**	**0.15**	**0.16**
SE	0.02	0.01	0.02	0.03	0.03	0.02	0.02	0.02
Range	0.05-0.25	0.13-0.24	0.01-0.26	0.08-0.28	0.00-0.23	0.06-0.23	0.04-0.28	0.11-0.23
Accepting Responsibility	**0.14**	**0.12**	**0.13**	**0.11**	**0.15**	**0.13**	**0.09**	**0.14**
SE	0.02	0.02	0.03	0.03	0.02	0.03	0.02	0.03
Range	0.03-0.22	0.03-0.21	0.00-0.21	0.00-0.19	0.05-0.23	0.00-0.25	0.02-0.17	0.00-0.29
Escape Avoidance	**0.09**	**0.07**	**0.10**	**0.07**	**0.10**	**0.06**	**0.09**	**0.08**
SE	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01
Range	0.02-0.14	0.01-0.14	0.04-0.17	0.00-0.12	0.06-0.13	0.01-0.12	0.05-0.17	0.03-0.15
Planful Problem Solving	**0.16**	**0.15**	**0.19**	**0.16**	**0.18**	**0.15**	**0.18**	**0.15**
SE	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.02
Range	0.07-0.26	0.05-0.22	0.10-0.26	0.12-0.25	0.11-0.29	0.06-0.24	0.07-0.30	0.08-0.25
Positive Reappraisal	**0.10**	**0.14**	**0.12**	**0.14**	**0.11**	**0.14**	**0.10**	**0.13**
SE	0.01	0.02	0.02	0.02	0.02	0.03	0.02	0.02
Range	0.05-0.17	0.07-0.19	0.02-0.21	0.03-0.22	0.05-0.18	0.02-0.30	0.02-0.18	0.04-0.23

Table 4.9 24-Hour Urinary Free Cortisol Concentrations (mean ± SE)

Urinary Free Cortisol (ug/24 hours)	Test 1		Test 2		Test 3	
	SS n = 9	CG n = 8	SS n = 9	CG n = 8	SS n = 9	CG n = 8
Concentration	**32.40**	**36.14**	**38.87**	**36.14**	**35.96**	**40.41**
SE	9.82	5.85	4.75	7.06	6.28	6.44
Range	8.93-101.11	7.69-49.96	16.93-54.56	9.01-73.31	9.59-56.39	23.37-80.14
Mean Urine Volume (mL)	**1050**	**934**	**923**	**981**	**1225**	**1023**
SE	194	121	145	132	188	100
Range	590-2090	500-1720	370-1730	340-1400	430-2040	520-1380

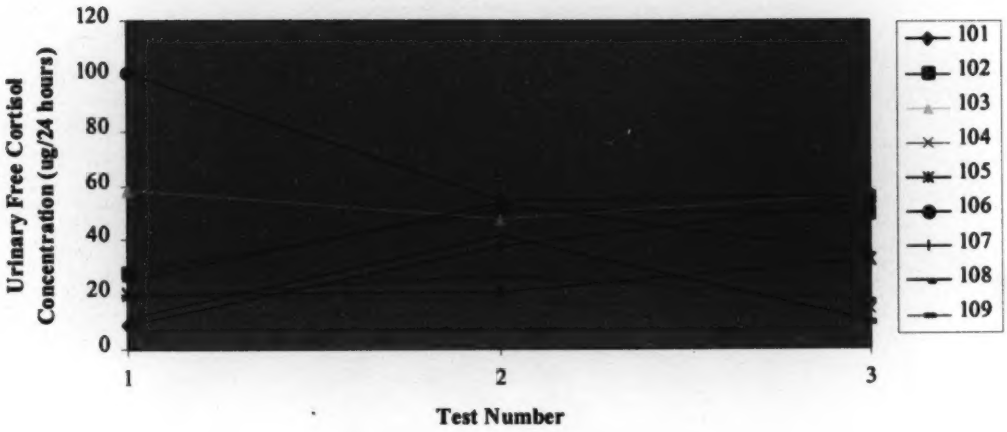


Figure 4.3 SS: Individual 24-hour urinary free cortisol concentrations (graphed by subject number)

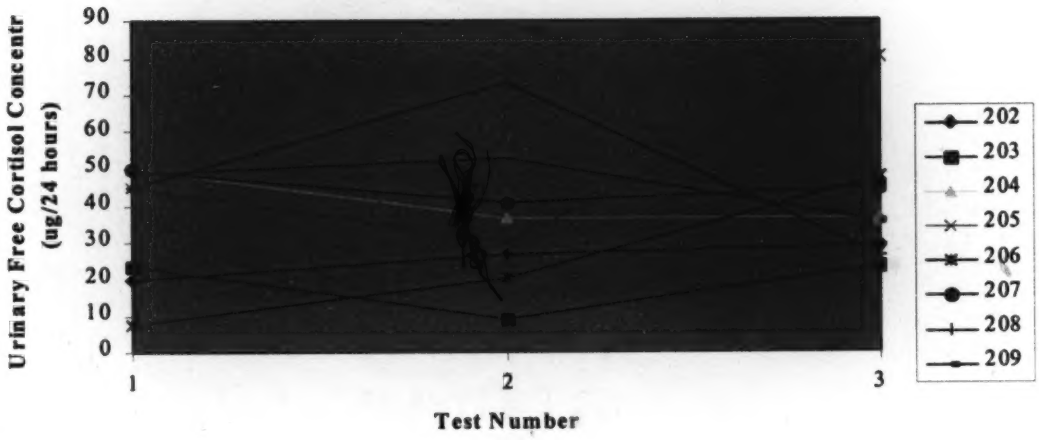


Figure 4.4 CG: Individual 24-hour urinary free cortisol concentrations (graphed by subject number)

Cortisol Assay Performance

Low and high level quality control (QC) values were run with each standard curve on both days of cortisol assays. The reported ranges were 3.1-4.7 µg/dL for the low level and 28.3-37.5 for the high level (DPC®, CON6®). Figures 4.5 and 4.6 show that the low

QC values from this study fall within the expected ranges on both testing days however, the high QC values on day 1 of the cortisol assay (27.3 µg/dL) were slightly lower than the low end of the expected range (28.3 µg/dL). The mean coefficients of variation (CV) for the duplicate QC values on both days were 3.9% for the low QC values and 5.0% for the high QC values.

Intra and inter assay performance were assessed. Coefficients of variation were calculated for each set of duplicate tubes and then a random sample of 5% of the total number of tubes was used to calculate the mean CV for that test day. This random sample excluded the high CV samples which were repeated on day 2, the quality controls, and the extraction efficiency tubes which were not used in urinary free cortisol calculations. The resulting mean intra-assay CV for day 1 was 8.0% and 9.3% for day 2. The mean of these 2 intra-assay CVs is 8.7%, which is the inter-assay CV for the cortisol assay in this study.

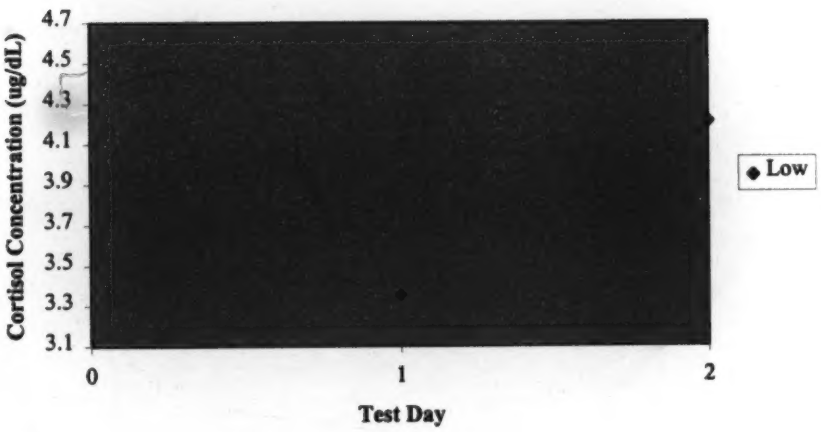


Figure 4.5 Low level quality control concentrations for both cortisol assay days

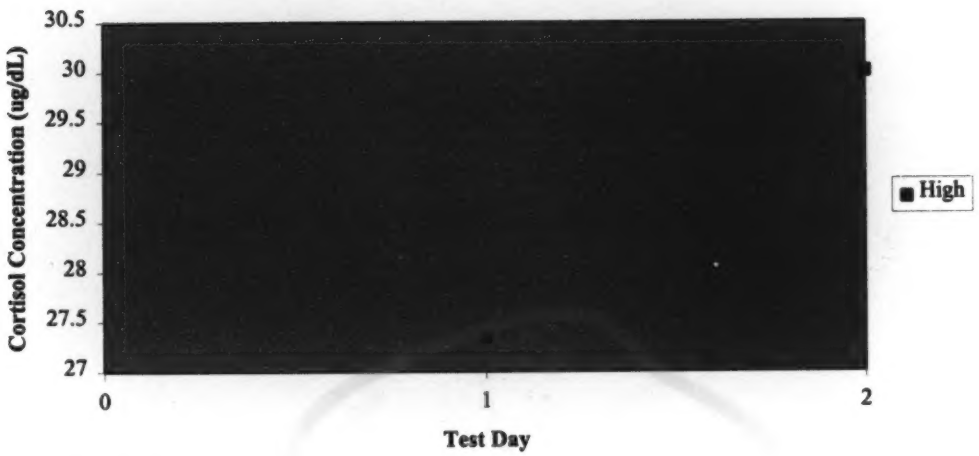


Figure 4.6 High level quality control concentrations for both cortisol assay days

Menstrual Cycle Characteristics

Table 4.10 shows the mean period length, cycle interval and PBAC score for each subject, as well as group means for each variable. Mean values were not statistically different between groups. Individual menstrual cycle characteristics are shown in Appendix S (SS) and Appendix T (CG). Subject 108 in the synchro group only had one menstrual period during the study. She did have duration and date of day 1 information for her last cycle (in July,1996), which was included as cycle 1 in Appendix S. Due to the late recruitment of the control group, there are not equal numbers of cycles between groups. One subject (206) in the control group did not submit any of her menstrual cycle information. She was excluded from the calculation of group means that are shown in Table 4.10.

Table 4.10 Mean Menstrual Cycle Information for each Subject and Group

ID #	Number of Cycles Recorded	Mean Length of period (days)	Mean PBAC Score	Mean Interval Between Cycles (days)
SS				
101	9	4.6	117	32
102	9	4.4	90	33
103	9	4.7	63	35
104	8	6.0	122	32
105	8	5.9	104	28
106	8	5.5	71	33
107	9	5.6	198	33
108	2	4.7	44	89
109	9	5.0	60	29
Mean	**8**	**5.2**	**97**	**38**
SE	**1**	**0.2**	**16**	**6**
CG				
201	6	6.3	168	34
202	6	4.5	95	35
203	6	5.3	225	30
204	7	5.3	301	26
205	5	6.0	67	30
206	0	n/a	n/a	n/a
207	5	5.8	85	36
208	4	4.8	51	32
Mean	**5**	**5.4**	**142**	**32**
SE	**1**	**0.2**	**35**	**1**

PBAC = Pictorial Blood Loss Assessment Chart
n/a = unable to compute mean due to missing data

Reproductive Hormones

Luteinizing Hormone

Mean LH pulse data are shown in Table 4.11. The repeated measures ANOVA demonstrated no statistically significant main effects of group or time, and no interaction effects. Individual LH pulse data are shown in Appendix U and individual LH graphs are shown in Appendix V. Technical problems with the intravenous catheter precluded a complete 8 hour testing session at one testing time in 2 of the control subjects [202 (test 1) and 206 (test 2)]. For subject 202, blood was drawn for 6.5 hours at the first testing

session and for subject 206, blood was drawn for 6.0 hours at the second testing session. The other testing sessions for both subjects were complete 8 hours sessions. The results for the incomplete sessions were analyzed using the available data.

Table 4.11 Luteinizing Hormone Pulse Characteristics (mean ± SE)

	Pulse Frequency (# of peaks)		Mean Pulse Interval (minutes)		Mean Pulse Amplitude (IU/L)		Mean Pulse Area		Mean Measured Level (IU/L)	
	1	2	1	2	1	2	1	2	1	2
SS n = 9										
Mean	2.6	2.1	296.85	371.41	3.02	3.57	240.26	286.99	12.65	12.36
SE	0.3	0.3	68.44	88.01	0.61	0.43	51.83	50.04	0.72	1.16
CG n = 8										
Mean	2.3	2.4	383.54	367.19	2.77	3.14	176.64	232.69	11.21	13.52
SE	0.4	0.5	96.29	81.80	0.49	0.61	49.23	46.14	0.69	1.46

1 = testing time 1
2 = testing time 2

Luteinizing Hormone Assay Performance

Due to the large number of serum samples analyzed for LH, low and high level QC values were run with the standard curve and either in the middle or at the end of the assay on each of the 11 testing days. The reported ranges for the QCs were17-21 mIU/mL for the low level and 122-168 mIU/L for the high level (DPC®, CON6®). Figures 4.7 and 4.8 show that all of the high level QC values fell within the expected range, yet all of the low QC values were 2-8 mIU/mL higher than the expected range. The mean coefficients of variation (CV) for the duplicate QC values over all testing days were 5.0% for the low QC values and 4.7% for the high QC values.

Table 4.12 shows the daily intra-assay CV as calculated by selecting a random 5% of all tubes analyzed on that testing day, again excluding the quality controls and the

samples with high CVs which were re-analyzed on the following testing day. Averaging the daily intra-assay CV's resulted in an inter-assay CV of 6.8% for LH. Day 11 demonstrates an intra-assay CV (17.5 %) above the acceptable range (6-15 %, Chard, 1987). This higher CV can be explained by the fact that the tubes assayed on day 11 were repeat assays on the tubes that had high CVs on day 10. All of these tubes except 1 pair (13.365 IU/L) contained serum samples with very low LH levels (2.501-8.532 IU/L). The low LH concentration and the relatively low number of samples assayed (40 samples in duplicate) explain the higher intra-assay CV on day 11.

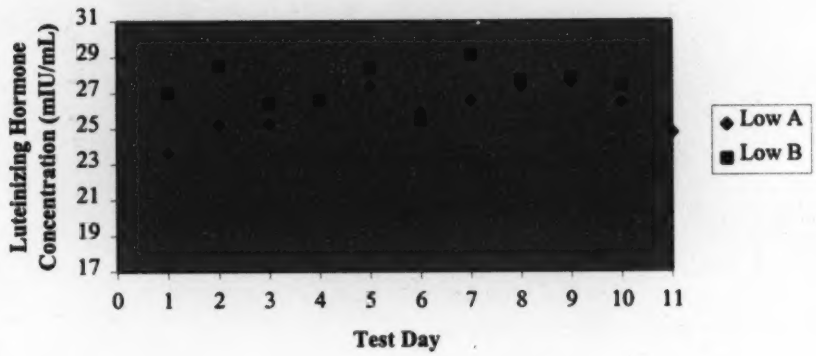


Figure 4.7 Low level quality control concentrations on all LH assay days

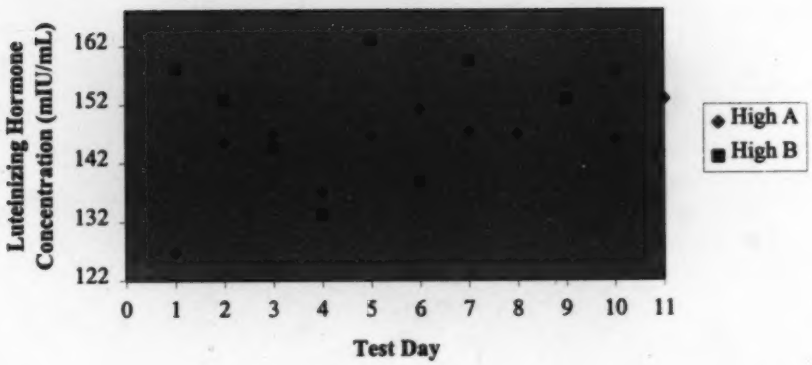


Figure 4.8 High level quality control concentrations on all LH assay days

Table 4.12 Daily Intra-assay Coefficient of Variation for LH

	Testing Day										
	1	2	3	4	5	6	7	8	9	10	11
Mean CV (%)	7.1	10.7	6.6	4.2	4.3	3.7	4.8	4.2	4.2	7.3	17.5

Progesterone

Methodological problems precluded a meaningful analysis of luteal phase progesterone in this study. The results obtained for each subject are presented in Appendix W. The difficulties associated with this test will be discussed in Chapter 5.

Correlational Statistics

Due to the large number of variables in this study, representative measures were chosen to best describe each independent variable for correlation purposes. Since each variable was tested a different number of times, a decision was made to examine correlations between chosen representative variables for pre and post-test results only in the following manner: the average of pre and post-test results (Table 4.13); the difference

between pre and post-test results; and pre and post-test results for each variable independently (see Appendix X for the full correlation matrix). The representative measures chosen were: mean LH concentration to represent the menstrual integrity (dependent variable), mean SOS to represent body fatness, mean cortisol concentration to represent stress, mean training hours per week to represent training, and mean energy intake to represent energy intake (independent variables). Surprisingly, the only statistically significant correlations in the matrix were: 1) SOS difference with hours per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 2) SOS average with hours per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 3) SOS at pre-test with training hours per week at pre-test ($r = -.658$, $p<.004$), 4) SOS at pre-test with training hours per week at post-test ($r = -.712$, $p<.001$), 5) SOS at post test with training hours per week at pre-test ($r = -.833$, $p<.000$), 6) SOS at post-test with training hours per week at post-test ($r = -.854$, $p<000$), 7) cortisol at pre-test with average training hours per week ($r = -.603$, $p<.010$), 8) cortisol at pre-test with the difference in pre-post training hours ($r = -.603$, $p<.010$), 9) calories at pre-test with cortisol at pre-test ($r = .484$, $p<.049$), 10) calories at post-test with cortisol at pre-test ($r = .506$, $p<.038$).

Table 4.13 Correlations of Representative Independent and Dependent Variables

	SOS (Body Fatness)	Cortisol Concentration (Stress)	Training Hrs/Week (Training)	Calories (Energy Intake)
Luteinizing Hormone Concentration	.335	-.329	.044	-.014

Chapter Five
Discussion

Overview

Energetic challenges (inadequate nutrition, low body fatness, and intense, extensive exercise training), stress (physical and psychologic), and a history of irregular menses are predisposing factors that have been associated with the development of menstrual disorders in female athletes. The causal mechanism which associates these predisposing factors to menstrual disorders has not been elucidated. To investigate this problem, the relationships among 24-hour urinary cortisol, energy intake, body composition, and training with the menstrual cycles of elite female synchronized swimmers were investigated in a 10-month prospective study.

The sport and the age of the subjects examined in this study were unique. The number of variables studied, the prospective design, the sedentary control group which was selected based on age and gynecologic age, and the standardization of the measures taken were also unique features of this study. Measures were standardized in the experimental group for training phase. Hormonal measures were standardized in both groups for time of day and LH and P measures were standardized for menstrual cycle phase.

The results indicated that the synchronized swimmers (SS) were statistically significantly taller, leaner, and had higher maximum oxygen consumption than the control group (CG). Significant differences between groups or testing times were not demonstrated for the other variables. Case studies revealed key individual differences.

The correlational statistics failed to show high correlations between any of the variables in this study. The details of these findings are discussed in the following pages.

Subject Characteristics

There were no statistically significant differences in age and gynecologic age between the synchronized swimmers (SS) and the control group (CG), thus the groups were well matched on these variables. This is important in this study which had relatively young subjects and where reproductive maturity had the potential to influence results. Height and weight were also not statistically significantly different between groups at the initial testing session. Body mass index (BMI), sum of skinfold (SOS), and Canadian Standardized Test of Fitness (CSTF) percentile results all indicated that the SS were significantly leaner than the CG at the initial testing session. This is not surprising since the SS were training 3-5 hours per day at the time of the initial testing session and were chronically trained whereas the subjects in the CG were sedentary. Both group mean BMI scores were within the healthy limits set for females 15-19 years old (CSTF Operations Manual, 1986). Ranges for BMI calculated from mean heights and weights in other studies using synchronized swimmers were 20.2-21.0 kg/m^2 (Erickson Gemma & Wells, 1987; Moffat, Katch, Freedson, & Lindeman, 1980; Heigenhauser, Oldridge, & Jones; Poole, Crepin, & Sevigny, 1980; Roby, Buono, Constable, Lowdon, & Tsao, 1983) which are similar to the mean BMI for the SS of 20.9 kg/m^2 found in the present study. The SOS and CSTF percentile results for the SS were within the healthy limits set for females 15-19 years, however the mean results for the CG fell in the health risk zone

(CSTF Operations Manual, 1986). This suggests that the CG had high adiposity despite being in the healthy zone for BMI.

Training

Typically a training season for a SS begins in late August. The training months of August, September, and October are characterized by high volume, long duration length swimming, running 3-4 times per week, weight training 3 times per week and basic synchro drills. Club tryouts are held in September and teams are selected in early October. October, November, and December are when the routines for the season are choreographed. During these months, the swimming volume is typically lower and the intensity is higher. Workouts tend to be more synchro specific at this time. The athletes generally have 10 days to 2 weeks off at the end of December. The competitive season begins in late January. Early January is usually focused on routine choreography revisions and synchro specific workouts. Choreography revisions are continued until early March at which time high intensity parts of the routines are repeated to increase the accuracy and to refine the execution. The second largest competition of the year is Western Divisionals which is held in late March. Following these championships, workouts are minimal and very routine specific. Many repeats of the routines are performed in early April to perfect the routine choreography. A training camp of 7-10 days is usually held in late April where the athletes are able to perfect their routines in preparation for the National Championships held in early May. Throughout the year, dryland workouts, landrill sessions, and weight training are adjuncts to the training regimen.

Maximum Oxygen Consumption (VO_2 max)

Given this yearly training snapshot, it is not surprising that the SS were more aerobically fit than the CG subjects, and that they did not increase their VO_2 max during the training season. First, these athletes were chronically trained when they entered the study. Second, at the time of the first VO_2 max tests, the SS were at the end of their high volume aerobic training phase, a time when the highest VO_2 max results would be expected. The longest routine in synchronized swimming is the team routine, which is a maximum of 5 minutes. Given this routine length, and given that approximately half of this time is spent underwater, synchronized swimming has a unique blend of energy system requirements, being both anaerobically and aerobically demanding. Synchronized swimmers must build their maximum oxygen consumption early in the season and then strive to maintain this aerobic fitness throughout the season while building the routine specific anaerobic fitness. Therefore, while an increase in aerobic fitness may be expected in untrained SS during a training season, the training regimen of a SS from November to May is focused on maintaining, not increasing, maximum oxygen consumption. The results obtained for the SS in this study are comparable to the VO_2 max results reported in other studies using synchronized swimmers (Poole et al., 1980; Roby et al., 1983).

Poole et al. (1980) studied a total of 32 synchronized swimmers consisting of 16 members of the 1977 Canadian National Team, 6 swimmers considered close to making the National Team and the 10 best junior swimmers in the country. Poole et al. used a

treadmill protocol to determine maximum oxygen consumption. Mean VO_2 max results for this group were 2.39 l/min (absolute), and 44.4 mL/kg/min (relative).

Roby et al. (1983) determined VO_2 max in a group of 13 national champion synchronized swimmers, also using a treadmill protocol. Their results showed a mean absolute VO_2 max of 2.43 l/min and a mean relative VO_2 max of 43.2 mL/kg/min.

In the present study, the SS were taller and heavier than the synchronized swimmers studied by Poole et al. (1980) and Roby et al. (1983). Absolute maximum oxygen consumption is higher in taller individuals (Jones, 1997; Plowman & Smith, 1997), which may partially account for the higher absolute VO_2 max (2.69 L/min) achieved by the SS in this study. However, in this study VO_2 max was determined using a cycle ergometer protocol which has been shown to elicit slightly lower results than treadmill protocols due to the smaller muscle mass involvement on a cycle (Maud & Foster, 1995). Treadmill measures of VO_2 max are typically 1.1-12 % higher than cycle ergometer VO_2 max measures (Kohrt, Morgan, Bates, & Skinner, 1987; Slievert & Wenger, 1993). Therefore, the SS in this study may have had even higher maximum oxygen consumption results than those reported here for absolute and relative VO_2 max (2.69 l/min and 45.11 mL/kg/min respectively) had they been tested on a treadmill. This potential improvement in the fitness level of synchronized swimmers in the past 15 years is reasonable since the sport has become more competitive internationally following its debut at the Olympic Games in 1984.

The control group had a mean VO_2 max of 31.65 mL/kg/min at test 1 and 35.34 mL/kg/min at test 2, both of which were below the expected value of 37 mL/kg/min for sedentary females 20-30 years old (Plowman & Smith, 1997). The slight improvement in VO_2 max in the control group between test 1 and test 2 was not statistically significant and may be explained by a learning effect since the first test was also the first time any of the CG subjects had ever been exposed to laboratory exercise testing.

Training Hours

In general, the training hours per week results indicate that the number of hours spent training remained fairly constant throughout the study, with the exception of October and December where lower mean training hours were seen. The 1996-1997 season was also unique for this group because there was a major international competition attended by 3 of the subjects in October. This competition would result in lower training hours per week for those athletes who attended the competition during the week that they were away and also during the week of their return when they had 3-5 days off, thereby lowering the group mean training hours for October (12.7 hours). December mean training hours (11.4 hours) were affected by the vacation time associated with Christmas. Some subjects maintained their running and weight training during their weeks off while others reported doing no training during this time. The mean training hours do not necessarily accurately reflect the true number of training hours for each SS. In addition to the team training hours each of the subjects also trained additional hours for solo routines, duet routines or both. Although these extra hours were calculated in the

group mean training hours the ranges reported reflect that all athletes did not train equal hours. Furthermore, with only 9 subjects, one subject missing several practices due to illness or injury could affect the group mean training hours.

Training Intensity

The mean training heart rates demonstrated in this study represent fairly low percentages of the athletes' maximum heart rates (54-65%). Training heart rates in synchronized swimmers have not been previously reported. The heart rates in this study were monitored for an entire 2-3 hour training session. Maintaining a higher heart rate for this time period would be challenging and may not be beneficial for some aspects of synchronized swimming. Figures, for example, require very slow, fluid movements. Figures are judged on technical design and on the control that the athlete exhibits while performing the figure. A high heart rate may not be conducive to the muscular control required to perform figures with a high degree of accuracy.

Gemma and Wells (1987) conducted a study of heart rates during synchronized swimming figures of various degrees of difficulty. They found that heart rates were typically between 75 and 130 beats per minute during figures (Gemma & Wells). These researchers found an increase in heart rate underwater during difficult movements followed by delayed bradycardia, and an unconscious induction of bradycardia in anticipation of facial immersion (Gemma & Wells). While the results of the present study do not attempt to correlate the exact timing of each element of synchronized swimming with a specific heart rate pattern, the low mean heart rates observed coincide

with the upper end of the heart rate range observed by Gemma and Wells. This is reasonable since heart rates were recorded from the SS in the present study not only while they did figures but during an entire synchronized swimming practice where routines, drills, and length swimming would also be included.

Synchronized swimming is a highly technical sport which requires a significant amount of partner work where one partner watches the other partner. For figures, as much as 1 hour of a 3 hour practice may be spent out of the water watching other team members. Since heart rate monitors recorded heart rates throughout the entire practice regardless of the tasks being performed, an hour of sitting on deck watching others would certainly lower the mean training heart rate for a practice.

Choreography and choreography revisions are also slow processes which are not typically physically challenging and may explain low heart rates during weeks and months (October) when choreography was a major focus. Furthermore, many of the SS reported that their heart rate receiver watches would often display zero when they were deep underwater. After downloading the heart rate data using the computer interface, it was evident that there were often large series of zeros. While these zeros were not used when calculating the mean training heart rate, they could potentially represent times when the athletes' heart rates were at their peak. Most months had similar mean heart rates with the exception of October and April. The low October heart rates are explained by the major routine choreography which occurred during this month. The fact that the April heart rates were lower than other months may reflect the fine technical detailing of

the routines prior to the National Championships which is time consuming but often not a situation where the heart rate would be consistently elevated. Low April heart rates may also be a result of the taper in training hours and volume of routine run throughs before Nationals.

Despite the fairly low percentages of the athletes' maximum heart rates reported, the athletes maintained their VO_2 max throughout the study. This indicates that the SS must have been training at a sufficient intensity and duration to maintain their fitness level.

Anthropometry

The SS were significantly taller than the CG at all testing times. Height is an advantage in synchronized swimming. Internationally, swimmers are performing their routines higher out of the water than in previous years. Swimmers are judged on not only what skills they perform but on how high out of the water they execute these skills. Taller swimmers, therefore, have an advantage because their longer limbs allow them to appear higher in the water. This judging trend may eventually lead to taller synchronized swimmers dominating the International podium.

During the summer months, the SS usually have 4-8 weeks away from synchro training. The duration of their time off depends on the summer competitions that they are selected to attend. Off-season training programs are prescribed for the SS and consist of running and weight training. Drop-in water sessions are also available to the athletes.

Therefore, while the SS are not sedentary during their time off, they train less than 10 hours per week, which is approximately half of their in-season training hours. As a result of this decrease in training hours over the summer, it is not surprising that the SS had significantly higher SOS and CSTF percentile scores in October (test 1) than they had at subsequent testing sessions.

The SS were leaner at all testing times than the CG. This is in contrast to earlier work which compared synchronized swimmers to non-athletic females where no differences were found between groups (Moffat et al., 1980; Roby et al., 1983). The sport of synchronized swimming has evolved considerably since these research projects were conducted over 15 years ago. Trends in synchro are now towards increased athleticism and power which reflect the introduction of teams into the Olympic Games in 1996. These trends would increase the physical demands on the athletes and perhaps lead to an overall reduction in body fat in synchronized swimmers as demonstrated in the present study.

Synchronized swimming presents unique challenges. Athletes must contend with the opposing forces of gravity and buoyancy while making every movement seem effortless. These unique physical challenges coupled with the challenges of aesthetics in a judged sport has led many of the top coaches to monitor the body fat of their athletes very closely. Other research has indicated that this close monitoring coupled with an emphasis on leanness for performance of aesthetic reasons might lead to pathological eating behaviours and decreased body weight and fat (Sundgot-Borgen, 1994). In the

present study, although eating behaviours were not examined, all of the SS were within the healthy ranges for body fat at all testing times. However, as demonstrated in a study of national team female synchronized swimmers (SS) and national team female field hockey (FH) players, being in the healthy range for BMI does not preclude disordered eating attitudes and behaviours (Marshall, Alentejano, Harber, McArger, & Ringrose, unpublished data). Marshall et al. demonstrated that SS scored significantly higher on 4 subscales of the Eating Disorder Inventory (EDI) than FH athletes. Individual EDI scores on 4 to 6 of the subscales for 2 of the SS were above the cutoff points established using data from clinically diagnosed eating disordered patients.

Significant main effects of time were observed for weight $F(3,45)=3.15$, $p<.05$ and BMI $F(3,45)=3.67$, $p<.05$. While post hoc multiple paired t-tests failed to show differences between testing times when the Bonferroni correction was used; significant differences at $p<.05$ were observed between mean weight at test 1 and 2, $p<.051$ and between mean BMI at test 1 and 2, 1 and 3, and 1 and 4, $p<.013$, .017, and .023, respectively. The Bonferroni correction was chosen to prevent a type I error.

Energy Intake

Both groups were below the daily energy intake recommended by Health Canada for females aged 16-18 years (2100 Calories) (Nutrition Recommendations, 1994) at all testing times except for the SS at test 1, who were slightly above this recommended caloric intake (2242.8 Calories). It is interesting that the SS who trained 3-5 hours per day did not consume significantly more calories or any macronutrient than the CG at any

testing time. It is difficult to explain how a physically active group can maintain their body weight and body fat, training 13-19 hours per week, while eating the same number of calories as a group with lower aerobic fitness that participates in no regular physical activity. It has been suggested that athletes who engage in high quantities of planned exercise may be less active than sedentary individuals during non-exercise periods (Horton, Drougas, Sharp, Martinez, Reed, & Hill, 1994). It is possible that while the CG did not participate in daily planned exercise, that they led an active lifestyle whereas the SS spent their hours training each day and then led a relatively sedentary lifestyle. This lifestyle difference could explain the weight maintenance with equal energy intake between groups despite the physical activity discrepancy. It is also possible that the SS have adapted metabolically to a reduced caloric intake where no weight loss is observed despite energy expenditure exceeding energy intake (Cumming et al., 1994; Dale & Goldberg, 1982; Laughlin & Yen, 1996).

Dale and Goldberg (1982) found that the female marathon runners in their study consumed only 474 more calories per day than the sedentary control group in spite of their exercise program which expended 500-1000 calories per day. Laughlin and Yen (1996) also found that despite an exercise energy expenditure of 900-1000 calories per day, the athletic women in their study had similar daily caloric intakes to the sedentary women in the study. Similar results in other studies led Cumming et al. (1994) to question the belief that if energy intake is held constant and energy output increased that weight will be progressively lost.

Others, however, do not support this theory that female athletes have an increased energetic efficiency (Horton et al. 1994). Horton et al. fed subjects fixed composition meals and closely monitored weight for 5 days. They also measured energy expenditure using a whole room indirect calorimeter for 24 hours on a training day and on a non-training day in a group of trained female cyclists (n=5) and in a sedentary female group (n=5). Their results showed that the energy balance between energy intake and energy expenditure was very close. They postulate that previous reports of higher energy efficiency in female athletes are based on an underestimation of true energy intake due to atypical eating habits during the data collection, underreporting, or a combination of both. Metabolic measurements were not made in this study, however there may be a possibility that the groups in this study were affected by societal pressures or that SS were affected by social pressures from a coach or sport subculture which may have caused them to underreport their energy intake. Further research where total daily energy expenditure is measured is needed to explain why athletes appear to have similar energy intake patterns to their sedentary peers, despite expending more energy.

The only statistically significant finding in the energy intake information was a main effect of time for grams of carbohydrate consumed $F(3,42)=2.89$, $p<.05$. While post hoc multiple paired t-tests failed to show differences between testing times when the Bonferroni correction was used; significant differences at $p<.05$ were observed between test 1 and test 2 ($p<.01$) and between test 1 and test 3 ($p<.029$). It should be noted that the Bonferroni correction is a conservative post hoc test that was chosen to avoid concluding that there is a difference when there is not one. The tendency toward higher

intake of carbohydrates at test 1 may indicate that the SS were responding to the energy demands of their high volume training phase by consuming more carbohydrates. This tendency may also indicate that some of the subjects were in the luteal phase of their menstrual cycle which has been demonstrated to elicit higher carbohydrate intake (Martini, Lampe, Slavin, & Kurzer, 1994).

The energy intake data in the present study may have been strengthened by controlling the energy intake recording for menstrual cycle phase. Energy intake records were recorded at standardized times in the SS, however these times were standardized to control for training variations and times when the subjects were in town, not menstrual cycle phase. Energy intake records were unable to be recorded at standardized times in the CG, other than stipulating that the days recorded include one weekend day and 2 weekdays, due to vacations and late recruitment into the study. There are sufficient data to support standardizing energy intake records for menstrual phase. Martini et al. (1994) found significant increases in energy, protein, carbohydrate, and fat intakes in the midluteal phase of the menstrual cycle when compared to the midfollicular phase. Tarasuk and Beaton (1991) also found significantly higher energy and fat intakes in the 10 premenstrual days compared to the 10 postmenstrual days. However, in the present study, subject recruitment and competition schedules precluded this standardization.

Stress

Derogatis Stress Profile (DSP)

The DSP results failed to demonstrate that the SS had higher stress levels than the CG. It was hypothesized that since the athletes had to contend with the additional pressures associated with training and competition that their stress level as measured by the scores in the 3 domains on the DSP, the total stress score, and the subjective stress score, would be higher . No significant differences were observed between groups on any of these measures. Perhaps the athletes did not perceive more stress than the CG, they may have had more effective coping strategies than the CG, or perhaps the athletes being chronically trained had adapted to the additional stressors of training and competition. It is also possible that the physical exercise allowed the athletes to dissipate any stress from other aspects of their life (Landers & Petruzzelo, 1994). Two of the subscales which comprise the domain scores on the DSP showed significant differences between groups. Attitude posture, a subscale in the personality mediators domain, was significantly higher in the SS than in the CG. Attitude posture refers to achievement ethic and the continuous drive toward new achievements (Derogatis, 1995). It is not surprising that a group of elite athletes had higher scores on this subscale than a group of sedentary individuals. Derogatis (1995) suggests that attitude posture is probably not as powerful a stress inducing factor as other personality traits. Health posture, a subscale in the environmental events domain, was significantly higher in the CG than in the SS. The questions that compose the health posture subscale are: 1) I rarely exercise, 2) I smoke

too much, 3) I am in good physical shape, 4) I don't take antacids for heartburn or gas, 5) I believe having good health is more important than anything, 6) I am very careful about my diet, and 7) I take tranquilizers to relax or sleep. Answers to these questions which would reflect negative health would produce high health posture scores. It is not surprising that the SS would consider themselves in good physical shape and would disagree that they rarely exercise (resulting in low health posture scores), whereas the CG would likely answer in the opposite direction.

It should be noted that some questions on the DSP are not applicable to younger subjects. On the questionnaire, respondents are asked questions about their work environment. When the subjects in this study were not employed, they were asked to answer the questions using school as their "job". Questions about the respondent's job loaded on vocational satisfaction, driven behaviour, domestic satisfaction, and relaxation potential subscales. Respondents are also asked 2 questions about their sex life: 1) I have a satisfying sex life and 2) Sex is an important part of life for me. These questions load on the domestic satisfaction and role definition subscales, respectively. When these was not applicable, respondents were instructed to record a zero which corresponds to "not at all true of me". Finally, since some of the subjects in this study were 15, the question which asks "when I am driving the car, I almost never rush through traffic" and loads on the time pressure subscale was also answered "not at all true of me" when the respondent did not drive. The impact of some subjects being unable to answer all of the questions on the DSP results is unknown.

The Ways of Coping Questionnaire (WOC)

No significant differences in coping styles were found between groups or between testing times on any of the 8 WOC subscales. This finding is interesting and reflects that coping styles may transcend very different lifestyles. Frequently used techniques included self-controlling (used 13-16 % of the time for coping with their selected issue), seeking social support (used 12-18 % of the time for coping with their selected issue), and planful problem solving (used 15-19 % of the time for coping with their selected issue). In addition, accepting responsibility was used 13-15 % of the time for coping with their selected issue by the SS at tests 2 and 3, and 14% of the time for coping with their selected issue by the CG at test 4. Coping is a process and varies in relation to the demands and constraints of the situation and may also vary as the situation unfolds (Folkman & Lazarus, 1988). For example, individuals tend to use more SC and escape avoidance, accept more responsibility, and seek less social support when faced with situations which are viewed as threatening to their self-esteem. However, when a loved one's well-being is threatened, individuals use more confrontive coping, distancing, escape avoidance and planful problem solving. Personalities also affect the type of coping strategy an individual chooses. Since the WOC questionnaire asks the respondent to pick a stressful event which occurred in the past week and answer all of the questionnaire based on the chosen situation, it would have been helpful to know the type of situation the subjects used at each testing session. Without this information, it is difficult to determine if the strategies chosen were appropriate. However, seeking social

support, accepting responsibility, planful problem solving, and positive reappraisal appear to be proactive coping strategies while confrontive coping, distancing, self-controlling, and escape avoidance may or may not be appropriate coping strategies depending on the situation. The results from this study demonstrate that 3 of the 4 coping strategies used by the subjects were positive. The fourth coping strategy, self-controlling may also be positive depending on the situation and the extent to which it was used.

Urinary Free Cortisol (UFC)

No statistically significant differences were found between groups for UFC. Both groups were within the expected ranges (20-90 μg/24 hours) for extracted UFC as described by INCSTAR in the Instruction Manual for the GammaCoat™ Cortisol ^{125}I RIA Kit. Figures 4.3 and 4.4 and the reported ranges demonstrate the wide individual variability in urinary cortisol concentrations over the 3 testing times. This wide variability may partially explain the lack of statistical significance in the UFC results. Wide inter-individual variation has also been reported in a cross sectional study where serum cortisol was monitored during prolonged exercise (Viru et al., 1992).

The SS were hypothesized to have higher UFC than the CG since athletes have been shown to have chronic hypercortisolism (Loucks et al., 1989). This hypercortisolism has been shown to approach the levels seen in patients with anorexia nervosa or depression (Gold et al., 1986a; Gold et al., 1986b). One possible explanations for the lower than expected cortisol levels is that a non weight-bearing sport like synchronized swimming may not produce the hypercortisolism reported in athletes who

participate in weight-bearing activities (Ding et al.1988; Loucks et al. 1989). The age of the subjects in this study may also explain the results. No normative data were found for athletes in the age range studied.

Menstrual Cycle Characteristics

Due to the discrepancies in the number of menstrual cycles documented during this study, ANOVA tests were not performed on the descriptive menstrual cycle data. Instead, statistical differences between group means were calculated using independent t-tests. No significant statistical differences were found between group means for any of the menstrual cycle characteristics.

The mean interval in days between subsequent menstrual periods in the SS is affected by Subject 108 who only reported 2 menstrual cycles during the study period (cycle 1 in Appendix S was a menstrual cycle that she reported having in July 1996, before the start of the study). When her data are excluded from the group mean, the SS and the CG have identical mean intervals (32 days). The interval between subsequent menstrual periods varied widely between and within individuals throughout the study. A between subject range in interval length of 22-104 days between cycles was observed in the SS (Appendix S). Only 2 SS subjects (105 and109) had consistent intervals (28-29 days and 26-32 respectively) for all 8 cycles recorded. All other SS had differences between the longest and shortest interval length ranging from 9-32 days. No consistent pattern was found in interval lengths. Some subjects had their highest intervals in the early months of the study, while some subjects had the highest intervals at the end of the

study. The CG had a between subject range in interval length of 20-46 days (Appendix T). The difference between the longest and shortest interval length for each individual member of the CG ranged from 5-25 days. This range is very large in a group that was screened to have "regular" menstrual cycles defined as menstrual cycles that occur consistently at intervals of 25-38 days (Loucks & Horvath, 1985). The young gynecologic age of both groups may account for these ranges. The mean interval lengths observed in this study are greater for the SS (38 days) and smaller for the CG (32 days) than the interval lengths reported in a study using synchronized swimmers and control subjects 18-22 years old (Ouellette et al., 1986). Ouellette et al. (1986) reported a mean interval length of 33.3 days and a range of 23-58 days for the synchronized swimmers (n=9) and a mean interval length of 35.1 days and a range of 24-92 days for the control group (n=40). The high variability in the interval length between subsequent menstrual periods may be a factor of the immature reproductive axis of some of the subjects in this study. In the SS, this high variability in menstrual cycle length may be early signs of exercise associated hormonal disturbances.

Menstrual period length remained consistent throughout the study in both groups. The SS had a mean flow duration of 5.2 days with a range of 4-7 days while the CG had a mean flow duration of 5.4 days with a range of 4-8 days. Vercellini, De Giorgi, Aimi, Panazza, Uglietti, and Crosignanai (1997) studied 152 (consisting of 59 women with a normal pelvis, 36 with nonendometriotic ovarian cysts, 29 with chronic pelvic inflammatory disease, and 28 with miscellaneous conditions; mean $\pm$ SD age 31.7 $\pm$ 4.3, 30.2 $\pm$ 6.3, 33.0 $\pm$ 4.5, 32.5 $\pm$ 5.5 years, respectively) premenopausal women without

endometriosis. The mean duration of menstrual flow for the women in that study was 4.6 days (Vercellini et al.), slightly shorter than the mean flow duration reported for both groups in the present study.

The mean PBAC score for the SS was 97 with a range of 23-287 while the CG had a mean PBAC score of 142 with a range of 52-301. While the CG had a tendency toward higher PBAC scores, a statistically significant difference was not found. The high variability in these scores may have precluded statistical differences between group means. PBAC scores are highly correlated with menstrual blood loss (Higham et al., 1990). Therefore, despite statisical insignificance, the control group had a tendency for higher mean menstrual blood loss than the SS since all of the SS except one had lower PBAC scores than the CG mean. Vercellini et al. (1997) used the PBACs and found that their nonendometriosis group had a mean score of 84 and a range of 56-129, lower than the mean scores reported in the present study. The absorbency of the specific sanitary products used by each subject in the present study may affect PBAC scores (Higham et al., 1990), however subjects likely used the same brand of products throughout the study which would control for the within subject variation in scores.

Reproductive Hormones

Luteinizing Hormone

The mean levels of LH in both groups are within the expected ranges (10-30 mIU/mL) for the early follicular phase as described in the INCSTAR Instruction Manual

for the GammaDab® LH ^{125}I RIA Kit. The LH levels and pulse characteristics in this study are similar to those reported in other studies. No significant ($p<.05$) differences were found between groups or testing times on any of the LH levels or pulse parameters.

Loucks et al. (1989) studied 3 groups of women; athletic women with amenorrhea (AA, n=9), athletic women with normal menstrual cycles (CA, n=9), and regularly cyclic sedentary women (CS, n=8) (in the early follicular phase). Blood samples were taken every 20 minutes from 0800 to 2000 h and every 10 minutes from 2000 until 0800 h the following morning. Mean serum LH levels for the wakeful hours were 9.8 IU/L, 13.4 IU/L, and 10.1 IU/L for the CS, CA, and AA groups respectively. The number of pulses per 8 hours during the wakeful period were 3.7, 2.9, and 2.1 for the CS, CA, and AA groups respectively. The pulse amplitudes were 4.2 IU/L, 6.5 IU/L and 3.9 IU/L for the CS, CA, and AA groups respectively.

Berga et al. (1989) studied 15 women (mean age = 24 years) with functional hypothalamic amenorrhea (FHA) and 16 normally menstruating (NM) women (mean age = 28 years) in the early follicular phase. These researchers collected blood samples every 15 minutes for 24-hours. The NM had 10.8 pulses per 24 hours as compared to 5.1 pulses per 24 hours in the FHA women. The NM had a mean pulse amplitude of 6.9 IU/L and the FHA had a mean pulse amplitude of 7.4 IU/L. The mean pulse intervals were 136 minutes and 235 minutes for the NM and the FHA women, respectively.

The mean LH levels for the SS in the present study (12.65 IU/L at test 1 and 12.36 IU/L at test 2) are slightly lower than those reported by Loucks et al. (1989) for CA

women (13.4 IU/L). The mean LH level for the CG (11.21 IU/L at test 1 and 13.52 IU/L at test 2) were slightly higher than the mean LH level reported by Loucks et al. (1989) for the CS women (9.8 IU/L).

The number of pulses per 8 hours in the present study were 2.6 at test 1 and 2.1 at test 2 for the SS and 2.3 at test 1 and 2.4 at test 2 for the CG. The SS numbers are very close to those reported by Loucks et al. (1989) for the CA women (2.9). The CG numbers are lower than those reported by Loucks et al. (3.7).

The LH pulse amplitude in both the SS (3.02 IU/L and 3.57 IU/L) and CG (2.77 IU/L and 3.14 IU/L) at testing times 1 and 2, respectively, were lower than those reported by Loucks et al. (1989) (4.2 and 6.5 IU/L for CS and CA, respectively).

The intervals between pulses were larger in the present study (296.85 minutes and 371.41 minutes for the SS; and 383.54 minutes and 367.19 minutes for the CG at tests 1 and 2, respectively) than in the data reported by Berga et al. (1989). The fewer pulses reported in the present study explain the longer pulse intervals than those reported by Berga et al. (1989) (136 and 235 minutes for the NM and FHA, respectively).

Berga et al. (1989) gave a description of the pulse parameters used to analyze the LH data in their study (a peak and nadir width of 2 and a t-statistic of 3.34 for both the up and down strokes). Loucks et al. (1989) did not report the pulse detection parameters that they used to interpret the LH data in their study. Differences in pulse parameters may explain the differences in pulse amplitude seen between the present study and the study

by Loucks et al. (1989). Comparisons of LH pulsatility between studies becomes difficult when the pulse parameters are not reported. Variability in sensitivity and the possibility of false positive pulse detection depend on the pulse parameters (peak and nadir width) and the t-statistic chosen (Veldhuis & Johnson, 1986).

Comparisons between the present study and other studies are also difficult because of the young chronological and gynecologic age of the subjects in the present study. The subjects in the Loucks et al. (1989) (mean age $\pm$ SD: 26.1 $\pm$ 4.2, 26.5 $\pm$ 2.7, 24.0 $\pm$ 4.5 years for the CS, CA, and AA groups respectively) and in the Berga et al. (1989) studies were older than the subjects in the present study.

The sensitivity of standard curve in the LH assay could have been improved by diluting the known serum standards provided in the assay which make up the standard curve and by eliminating the 150 IU/L in addition to the 300 IU/L standard which was eliminated from the protocol. The highest serum LH concentration found in this study was 40 IU/L therefore, the serum concentrations which comprise the standard curve could have been diluted which would have shifted the standard curve to the left and increased the sensitivity of the curve. Increasing the sensitivity of the standard curve allows for subtle differences between samples to be detected at low concentrations.

Progesterone

The methodological or technical problems associated with the progesterone analysis are of unknown origin. Two different batches of Salivettes® were used in this

study. Upon inspection of the progesterone results that were obtained, it appeared that the oldest batch of Salivettes®, which were used in the latter part of the study, elicited higher progesterone concentrations. Correspondence with Sarstedt, the Salivette® supplier, revealed that the composition of the Salivette® tube changed from polystyrene to polypropylene between the batches used in the present study. The effect that this tube composition would have on the storage of saliva in the tubes is unknown. The composition of the cotton wool swab within the Salivette® has not changed between tube batches. The Salivettes® were used within the 36 month shelf life that the manufacturer recommends for this product. One research article was found which discusses the problems with salivary 17-hydroxyprogesterone determinations using Salivettes® (Kruger, Breunig, Biskupek-Sigwart, & Dorr, 1996). These researchers report that salivary 17-hydroxyprogesterone analyzed from saliva contained in Salivettes® was significantly higher by 40 ng/l than saliva obtained without a Salivette® (Kruger et al., 1996). They speculate that the cotton wool in the Salivette® may cross-react with the antibody in the assay or affect binding affinity (Kruger et al., 1996). These findings do not explain the results in the present study since the concentrations observed varied widely and were not simply elevated from expected values. Freezer degradation of the saliva samples is not a viable explanation since the final 2 testing times were only one month apart in many cases. The progesterone assay also does not offer a viable explanation of the results obtained. All samples from the same subject were analyzed on the same testing day by the same researcher. Furthermore, all assay kits used were from the same lot number. All of the 16 quality controls except 2 which were run on the 6

days that the progesterone assay was conducted were within the expected ranges. This provides evidence that the assay was likely not the source of error. If the problems were associated with the way the tubes were stored in the subjects' freezers, the results would likely show more consistency within the same subject and less consistency between subjects. Despite extensive efforts to reveal the nature of the methodological or technical problems with the saliva collection and the progesterone assay in this study, the exact cause of the spurious results obtained remains unknown.

Correlational Data

The 10 areas where significant correlations occurred were: 1) SOS difference with hours per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 2) SOS average with hours per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 3) SOS at pre-test with training hours per week at pre-test ($r = -.658$, $p<.004$), 4) SOS at pre-test with training hours per week at post-test ($r = -.712$, $p<.001$), 5) SOS at post test with training hours per week at pre-test ($r = -.833$, $p<.000$), 6) SOS at post-test with training hours per week at post-test ($r = -.854$, $p<000$), 7) cortisol at pre-test with average training hours per week ($r =-.603$, $p<.010$), 8) cortisol at pre-test with the difference in pre-post training hours ($r = -.603$, $p<.010$), 9) calories at pre-test with cortisol at pre-test ($r = .484$, $p<.049$), 10) calories at post-test with cortisol at pre-test ($r = .506$, $p<.038$).

The first 6 significant correlations are logical. Body fat is often negatively correlated with the number of hours spent training. When training hours are increased,

presumably energy expenditure is also increased. Theoretically, if more calories are expended than taken in, a negative energy balance is created and weight and/or body fat are lost (Williams, 1995). The SS in this study demonstrated this as their training hours increased after the summer vacation, their body fat decreased between the first and second testing sessions. This decrease in body fat was then maintained with the training hours which remained relatively constant throughout the study.

The seventh and eighth correlations are more difficult to explain. Cortisol has been shown to be elevated in athletic populations (Loucks et al., 1989), however why only pre-test cortisol would be correlated with training hours and why this would be a negative correlation is unknown. Changes in caloric intake during times of stress have been reported (Schweiger et al., 1988). Schweiger et al. (1988) reported lower caloric intake was associated with higher levels of reported stress. This relationship may explain the correlation between caloric intake and cortisol, but not the direction of this correlation. Furthermore, why only pre-test cortisol is correlated with caloric intake is unknown since the cortisol levels at the pre-test were not significantly different from the cortisol levels at the other testing times.

Given the literature suggesting interrelationships among energy intake, training, cortisol levels, and body fat, the limited and puzzling correlations between the variables in this study are surprising. It is possible that the measures chosen to represent each variable were not appropriate indicators of that variable. For example, luteal phase progesterone may be a key indicator of menstrual dysfunction in athletes (Loucks, 1990)

and could therefore be highly correlated with stress, training, energy intake and possibly body fat. However because this measure was not available to represent menstrual function in the correlation matrix, perhaps important relationships among the variables in this study were missed. Further studies with larger sample sizes may explain the correlations observed here.

Case Studies

To extract individual differences from the group data, 4 synchronized swimmers were selected to examine individually as case studies. These swimmers were all members of the senior team and were chosen based on superior athletic performance (subjects 101, 103, and 109) and on outlying data (subject 108).

Synchronized Swimmer 101

Subject 101 was 18.7 years of age as of January 1, 1997, and her gynecologic age was 4.3 years, making her slightly older and more gynecologically mature than the SS group mean.

Maximum oxygen consumption results indicated that this subject was more aerobically fit [absolute VO_2 max = 2.71 l/min (test 1), 2.75 l/min (test 2); relative VO_2 max = 45.5 mL/kg/min (test 1), 47.4 mL/kg/min (test 2)] than the SS group mean at both testing times.

Subject 101 had been training in the sport of synchronized swimming for 9 years as of May 1, 1997. She trained 11 months of the year, 6 days, and an average of 19.4

hours per week. Her mean training heart rate was 120.4 bpm which is equivalent to 64% of her maximum heart rate. This athlete has competed both nationally and internationally.

This athlete was taller [176.4 cm (test 1), 175.1 cm (test 2), 175.1 cm (test 3), 175.3 cm (test 4)] and weighed less [59.5 kg (test 1), 57.3 kg (test 2), 59.3 kg (test 3), 57.8 kg (test 4)] than the SS group means at all testing times. BMI and SOS for this subject [19.1 kg/m^2, 55.4 mm (test 1), 18.7 kg/m^2, 52.1 mm (test 2), 19.3 kg/m^2, 54.9 mm (test 3), 18.8 kg/m^2, 49.0 mm (test 4)] were lower than the SS group mean at all testing times. CSTF percentile was higher [60 (test 1), 70 (test 2), 65 (test 3), 75 (test 4)] than the SS group mean at all testing times. BMI, SOS and CSTF percentile remained in the healthy zone for females aged 15-19 years at all testing times (CSTF Operations Manual, 1986).

This subject was exceptionally conscientious about recording her energy intake and providing labels and nutritional information for each food item consumed. Total calories consumed were higher [2249 (test 1), 2025 (test 2), 1982 (test 3), 2299 (test 4)] than the SS group mean at all testing times. Her protein intake as a percentage of total calories [13 % (test 1), 18 % (test 2), 15 % (test 3), 17 % (test 4)] was higher than the SS group mean at all testing times except test 1 where her results approximated the SS group mean. Grams of protein consumed [76 g (test 1), 94 g (test 2), 74 g (test 3), 103 g (test 4)] were considerably higher than the SS group mean at all testing times except test 1 where her results were lower than the SS group mean. Her intake of carbohydrates [63 % (test

1), 50 % (test 2), 59 % (test 3), 55 % (test 4)] was lower as a percentage of the total calories consumed than the SS group mean. Yet, her total grams of carbohydrates consumed [370 g (test 1), 263 g (test 2), 288 g (test 3), 322 g (test 4)] were higher than the SS group mean at all testing times except test 2 where her results were lower than the group mean. Intake of fat as of a percentage of caloric intake and as total grams consumed [21 %, 51 g (test 1), 28 %, 66 g (test 2), 26 %, 58 g (test 3), 28 %, 75 g (test 4)] were higher than the SS group mean at all testing times except test 1 where the percentage of the total calories consumed that were fat was approximately equal to the SS group mean and the grams of fat consumed were lower than the SS group mean.

DSP results showed that the attitude posture scores were higher for this subject at tests 3 and 4 (17 and 17 respectively). This subscale has high stress-inducing potential (Derogatis, 1995), and refers to achievement ethic. It is expected that closer to the National Championships, an elite SS would be more driven toward achievement. All other subscales had fairly consistent scores throughout the study and showed no consistent pattern for the small variations that did exist. The personality mediators domain score is comprised of those traits that have demonstrated etiological relevance to one or more stress-related disorder (Derogatis, 1995). This subject had personality mediators scores which ranged from 193-222 with the highest score recorded at test 3. All of her scores in this domain were lower than the SS groups means. The environmental events domain measures the stress which confronts us in our primary environments and therefore assesses stress with respect to home, work and healthy lifestyle. This subject had environmental events scores which ranged from 129-139 with

the highest score being recorded at test 1. Again, this subject scored lower in this domain than the SS group means at all testing times. The final domain is the emotional response domain which measures hostility, anxiety and depression which are essentially universal indicators of emotional distress (Derogatis, 1995). This subject had a range of scores in the emotional response domain from 145-165 with the highest scores being recorded at test 3 (165) and test 4 (160). This subject had higher emotional response scores than the SS group mean at all testing times except at test 2 (145). Again the higher scores at the end of the training season may represent performance anxiety in anticipation of the major competitions. The subjective stress score (SSS) is a simple test where the subjects mark an "X" on a line to indicate their perceived stress level. An "X" closer to the right end of the line would indicate a higher stress level than an "X" closer to the left end of the line. This subject's scores ranged from 48-75 and were higher than the SS group mean at all testing times except at test 4 (48). The high scores on tests 2 and 3 (77 and 75 respectively) may reflect some concerns that this subject had about her duet partner who had some health problems during this time and eventually had to drop out of the duet before the final testing session which may have resulted in a feeling of relief for this subject. The Total Stress Score (TSS) is an overall summary of the individual's DSP stress measurement. This subject had TSSs which ranged from 467-519 with the highest score being recorded at test 3. Again, this score may reflect the challenges that her duet partner's health presented to this subject. The TSS for this subject was lower than the SS group mean scores at all testing times. Derogatis (1995) suggests that the discrepancy between T-scores of the SSS and the TSS in both magnitude and direction can reflect

stress management. If the SSS is within 8 points of the TSS then the individual is considered to have an accurate perception of their stress. If the difference between these scores in greater than 8, then the individual is considered to be in "stress augmentation" (perceiving more stress than the DSP scores indicate) or "stress denial" (perceiving less stress than the DSP scores indicate) depending on the direction. The T-scores for SSS and TSS [50, 40 (test 1); 58, 36 (test 2); 57, 43 (test 3); 45, 40 (test 4)] for this subject indicate that she augmented her stress level at all testing times except test 4 where her perception of her stress level was accurate.

The WOC results were analyzed on an individual basis by highlighting the top 3 coping strategies used by each athlete at each of the testing sessions. This athlete used seeking social support [0.16 (test 2), 0.23 (test 3), 0.18 (test 4)] and positive reappraisal [0.16 (test 1), 0.14 (test 2), 0.16 (test 3)] as 2 of her top 3 coping strategies relatively more frequently than other coping strategies. Planful problem solving was used as one of her top strategies at test 1 (0.17) and test 4 (0.18) while confrontive coping [0.20 (test 3)], self-controlling [0.14 (test 2)], accepting responsibility [0.18 (test 1)], and escape avoidance [0.12 (test 3] were used as one of her top 3 strategies only at one testing time.

The UFC concentration [8.93 μg/24 hours (test 1), 37.50 μg/24 hours (test 2), 53.19 μg/24 hours (test 3)] for this subject rose throughout the study. This is consistent with the hypothesis that the stress level in the SS would rise throughout the training year and peak at the National Championship. The UFC at test 1 was below the expected 24-hour UFC concentration (20-90 μg) for extracted urine samples. This low concentration

may represent the relatively low training intensity and competitive stress level in the early part of the training season.

The menstrual cycle characteristics for this subject (see Appendix S) showed that the interval between her menstrual periods became longer as the training year progressed. The shortest interval was for cycle 1 (29 days) and the longest interval was for cycle 9 (39 days). The flow duration of her menstrual periods remained consistent at 4-5 days throughout the study. No consistent trend was found in the PBAC scores with the highest score being at cycle 2 (151) and the lowest score being at cycle 8 (79).

Subject 101 had 3 LH pulses at both tests 1 and 2. This pulse frequency is higher at both testing times than the SS group mean. The mean pulse interval was longer at test 2 (136.00 minutes) than at test 1 (170.00 minutes) and was longer than the SS group mean. Mean pulse amplitude and mean pulse area and mean measured level were also lower at test 2 (2.56 IU/L, 222.78, 11.04 IU/L) than at test 1 (5.19 IU/L, 494.45, 14.23 IU/L). When compared to the SS group means, mean pulse amplitude, mean pulse area, and mean measured level for subject 101 were higher at test 1 and lower at test 2.

This subject's results portray the hypothesized overview of a typical training year for a SS. During the training year, her maximum oxygen consumption, weight, body fat, and energy intake remained relatively stable. This is expected in a chronically trained elite athlete. Urinary free cortisol increased as this athlete came closer to the National competition. The interval between subsequent menstrual periods increased throughout the study and LH pulse amplitude, area, and mean level decreased. This pattern of

lengthening menstrual cycles has been demonstrated in other prospective studies (Bullen et al., 1985; Beitins et al., 1991).

Synchronized Swimmer 103

Subject 103 was 16.0 years of age as of January 1, 1997; her gynecologic age was 2.1 years at this time making her slightly younger and less gynecologically mature than the SS group mean.

Maximum oxygen consumption results indicated that this subject was more aerobically fit [absolute VO_2 max = 3.71 l/min (test 1), 3.90 l/min (test 2); relative VO_2 max = 47.4 mL/kg/min (test 1), 51.6 mL/kg/min (test 2)] than the SS group mean at both testing times.

Subject 103 had been training in the sport of synchronized swimming for 10 years as of May 1, 1997. She trained 11 months of the year, 6 days and an average of 19.4 hours per week. Her mean training heart rate was 110.7 bpm which is equivalent to 58% of her maximum heart rate. This athlete has competed both nationally and internationally.

This athlete was taller [184.6 cm (test 1), 184.7 cm (test 2), 183.6 cm (test 3), 183.3 cm (test 4)] and weighed more [78.0 kg (test 1), 76.9 kg (test 2), 78.1 kg (test 3), 75.4 kg (test 4)] than the SS group means at all testing times. Her BMI [22.9 kg/m^2 (test 1), 22.5 kg/m^2 (test 2), 23.2 kg/m^2 (test 3), 22.4 kg/m^2 (test 4)] was higher than the SS group mean at all testing times. SOS for this subject [64.6 mm (test 1), 57.6 mm (test 2), 54.2 mm (test 3), 50.7 (test 4)] were lower than the SS group mean at all testing times

except test 2 where her results were higher than the SS group mean. Her SOS results decreased progressively throughout the year. CSTF percentile increased throughout the year [40 (test 1), 55 (test 2), 65 (test 3), 70 (test 4)]and was lower at the tests 1 and 2 than the SS group mean and higher at tests 3 and 4 than the SS group mean. BMI, SOS and CSTF percentiles remained in the healthy zone for females aged 15-19 years at all testing times (CSTF Operations Manual, 1986). Weight, BMI, and SOS were all lowest at test 4. CSTF percentile was highest at test 4. It is logical that in the final preparatory stage before the National Championships that this athlete would be at her lightest and leanest.

Total calories consumed were [2009 (test 1), 2154 (test 2), 2152 (test 3), 1970 (test 4)] higher at test 2 and test 3 and lower at test 1 and 4 than the SS group mean. At all testing times, this athlete had lower caloric intake per kilogram body weight per day [25.76 (test 1), 28.01 (test 2), 27.55 (test 3), 26.13 (test 4)] than the 45-50 kcal/kg/day recommended by Economos, Bortz, and Nelson (1993) for female athletes training for more than 90 minutes per day. The protein intake as a percentage of total calories [17 % (test 1), 18 % (test 2), 15 % (test 3), 19 % (test 4)] for this subject was higher than the SS group mean at all testing times. Grams of protein consumed [86 g (test 1), 104 g (test 2), 83 g (test 3), 89 g (test 4)] were considerably higher than the SS group mean at all testing times. However, this athlete's protein intake expressed per kilogram body weight per day [1.1 (test 1), 1.4 (test 2), 1.1 (test 3), 1.2 (test 4)] was within the recommended protein intake of 1.1-1.5 g/kg/day for females participating in aerobic sports (Economos et al.). Her intake of carbohydrates [55 % (test 1), 55 % (test 2), 54 % (test 3), 50 % (test 4)] was lower as a percentage of the total calories consumed than the SS group mean and lower

than the 70 % of total calories recommended for athletes (Economos et al.). Her total grams of carbohydrates consumed [268 g (test 1), 296 g (test 2), 305 g (test 3), 246 g (test 4)] were higher at test 2 and 3 and lower at tests 1 and 4 than the SS group mean. Intake of fat as of a percentage of caloric intake and as total grams consumed [28 %, 70 g (test 1), 27 %, 68 g (test 2), 31 %, 76 g (test 3), 31 %, 68 g (test 4)] were higher than the SS group mean at all testing times and higher than the recommended fat intake of less than 25 % of total calories for athletes on low energy diets (<2200 Calories/day). Therefore, this athlete is consuming less calories and carbohydrate and more fat than the recommended intakes (Economos et al.). Her protein intake was within the recommended range (Economos et al.)

Derogatis Stress Profile (DSP) results showed that the Vocational Environment scores for this subject ranged from 7-17 and the lowest score for this subject was recorded at test 3. When the subjects did not have a job, they were instructed to treat school as their job. For this subject, a high school student, test 3 would have been just after her January exams which would explain her low school stress level at this point in her school year. Health posture scores ranged from 2 to 7 with the highest score (7) being recorded at test 1. This can potentially be explained by the fact that this athlete may have perceived her fitness level to be lower at the first testing session than at subsequent testing sessions. All other subscales had fairly consistent scores throughout the study and showed no consistent pattern for the small variations that did exist. This subject had personality mediators domain scores which ranged from 244-259 with the highest score recorded at test 4. All of her scores in this domain were higher than the SS groups means.

This subject had environmental events domain scores which ranged from 133-157 with the highest score being recorded at test 1. This subject scored higher in this domain than the SS group means at tests 1(157) and 4 (151) and lower than the SS group mean at tests 2 (142) and 3 (133). This subject had a range of scores in the emotional response domain from 128-156 with the highest scores being recorded at test 4. This subject had higher emotional response scores than the SS group mean at tests 1 (155) and 4 (156) and lower scores than the SS group mean at tests 2 (128) and 3 (143). The higher score at the beginning may reflect anxiety about club tryouts and training resumption while the high score at the end may represent performance anxiety in anticipation of the major competitions. The SSS for this subject ranged from 62-79, with the highest score being recorded at test 3. Her SSS were higher than the SS group mean at all testing times. This subject had TSSs which ranged from 514-566 with the highest score being recorded at test 4. The TSSs for this subject were higher than the SS group mean at test 1 (562) and test 4 (566) and lower than the SS group mean at test 2 (514) and 3 (521). The T-scores for SSS and TSS [51, 49 (test 1); 51, 43 (test 2); 60, 44 (test 3); 57, 50 (test 4)] for this subject indicate that she had an accurate perception of her stress at all testing time except test 3 where she augmented her stress level.

This athlete used accepting responsibility [0.18 (test 1), 0.20 (test 2), 0.23 (test 3)] and planful problem solving [0.21 (test 1), 0.16 (test 2), 0.17 (test 3)] as 2 of her top 3 coping strategies relatively more frequently than other coping strategies. Other strategies used as her top 3 methods of coping were confrontive coping [0.19 (test 2), 0.25 (test 4)],

self-controlling [0.16 (test 3), 0.16 (test 4)], and seeking social support [0.16 (test 1), 0.14 (test 4)].

The UFC concentration [58.10 µg/24 hours (test 1), 47.44 µg/24 hours (test 2), 56.39 µg/24 hours (test 3)] for this subject remained relatively consistent throughout the study. This may reflect a high intensity approach to daily training, or advanced coping techniques to confront the stress of the competitive season. All UFC concentrations for this subject were within the expected range for 24-hour extracted urine samples.

The menstrual cycle information revealed that this subject had relatively consistent intervals between her menstrual periods for the first 7 cycles of the study (29-34 days). However between cycles 8 and 9 the interval increased to 61 days. The athletes left for the National Championships on April 25, 1997 and returned on May 5, 1997. This subject had 1 menstrual period starting on April 7, 1997 (cycle 8) and the subsequent period started June 1, 1997 (cycle 9), thus she did not menstruate during the 7 day training camp in Arizona (April 13-19), the National Championships, or the 3 weeks following the National Championships. It is unfortunate that cortisol, LH and energy intake measures were not taken during this time frame since these measures may help to clarify the origin of the sudden increase in menstrual cycle interval length. The menstrual flow duration remained consistent (4-6 days) throughout the study for this subject. The PBAC scores for this subject (34-87) were lower than those for other subjects indicating light menstrual flow.

Subject 103 had 3 LH pulses at test 1 and 2 at test 2. This pulse frequency is higher at test 1 and lower at test 2 than the SS group mean. The mean pulse interval was longer at test 1 (205.00 minutes) than at test 2 (290.00 minutes) and longer than the SS group mean at both testing times. Mean pulse amplitude and mean pulse area were lower at test 1 (1.40 IU/L, 138.98) than at test 2 (3.30 IU/L, 188.96); these values were lower than the SS group means at both testing times. The mean measured level of LH for this subject was 13.10 IU/l at test 1 and 12.80 IU/l at test 2. These values were higher than the SS group mean at both testing times.

This athlete increased her maximum oxygen consumption and decreased her weight and body fat during this study. Her lowest body weight and body fat results coincided with her lowest caloric intake and her longest menstrual cycle length. This pattern could indicate that when faced with an energy intake shortage, this athlete's body may have sacrificed reproductive function (menstrual cycle length) and fat storage in order to divert the available energy to more essential processes (Wade et al., 1996).

Synchronized Swimmer 108

Subject 108 was 18.6 years of age as of January 1, 1997; her gynecologic age was 0.9 years at this time making her slightly older but much less gynecologically mature than the SS group mean.

Maximum oxygen consumption results indicated that this subject had slightly lower absolute VO_2 max results [2.41 l/min (test 1), 2.49 l/min (test 2)] than the SS group

mean at both testing times. This may be a result of this subject being shorter than the SS group mean since height is directly related to absolute VO_2 max (Jones, 1997). Due to the low body weight of this subject, her relative VO_2 max results [49.4 mL/kg/min (test 1), 50.5 mL/kg/min (test 2)] were higher than the SS group mean at both testing times.

Subject 108 had been training in the sport of synchronized swimming for 3 years as of May 1, 1997. Her mean training heart rate was 109.3 bpm which is equivalent to 59 % of her maximum heart rate. She trained 10 months of the year, 6 days and an average of 17.1 hours per week. This athlete has competed at the Provincial and Western Canadian level and was one of the weakest synchronized swimmers in this study. Prior to her competing as a synchronized swimmer, this subject had trained as a competitive swimmer for 4 years.

This athlete was shorter [163.8 cm (test 1), 164.7 cm (test 2), 165.4 cm (test 3), 165.6 cm (test 4)] and weighed less [48.7 kg (test 1), 46.9 kg (test 2), 48.4 kg (test 3), 49.2 kg (test 4)] than the SS group means at all testing times. Her BMI [18.2 kg/m^2 (test 1), 17.3 kg/m^2 (test 2), 17.7 kg/m^2 (test 3), 17.9 kg/m^2 (test 4)] was lower than the SS group mean at all testing times. SOS for this subject [50.0 mm (test 1), 43.3 mm (test 2), 45.9 mm (test 3), 48.5 (test 4)] were also lower than the SS group mean at all testing times. CSTF percentile [75 (test 1), 85 (test 2), 80 (test 3), 75 (test 4)] was higher throughout the study than the SS group mean. BMI results at each testing time placed this subject in the health risk zone for females 15-19 years old for being too lean (CSTF Operations Manual, 1986). SOS and CSTF percentile results also placed this subject in

the health risk zone for females aged 15-19 years at test 2 again for being too lean (CSTF Operations Manual, 1986).

Total calories consumed [1763 (test 1), 973 (test 2), 1060 (test 3), 1907 (test 4)] were lower than the SS group mean at all testing times. The protein intake as a percentage of total calories [9 % (test 1), 10 % (test 2), 12 % (test 3), 10 % (test 4)] for this subject was lower than the SS group mean at all testing times. Grams of protein consumed [40 g (test 1), 25 g (test 2), 30 g (test 3), 46 g (test 4)] were considerably lower than the SS group mean at all testing times. Her intake of carbohydrates was higher as a percentage of the total calories consumed [82 % (test 1), 78 % (test 2), 74 % (test 3), 66 % (test 4)] than the SS group mean. Her total grams of carbohydrates consumed [372 g (test 1), 197 g (test 2), 207 g (test 3), 325 g (test 4)] were higher at test 1 and 4 and lower at tests 2 and 3 than the SS group mean. Intake of fat as of a percentage of caloric intake and as total grams consumed [10 %, 20 g (test 1), 11 %, 13 g (test 2), 14 %, 17 g (test 3), 24 %, 52 g (test 4)] were much lower than the SS group mean at all testing times.

Derogatis Stress Profile (DSP) results showed that this subject recorded her lowest stress scores for 4 (attitude posture, relaxation potential, role definition, and vocational environment) of the 11 subscales on the final test. This may reflect the fact that this subject did not attend Nationals in 1997 and therefore was not facing the same magnitude of competitive stress as the other athletes at the time of test 4. All other subscales had fairly consistent scores throughout the study and showed no consistent pattern for the small variations that did exist. This subject had personality mediators

domain scores which ranged from 209-249 with the highest score recorded at test 2. Her scores in this domain were higher than the SS group means at tests 2 (249) and 4 (241) and lower than the SS group means at tests 1 (234) and 3 (241). This subject had environmental events domain scores which ranged from 128-153 with the highest score being recorded at test 3. This subject scored lower in this domain than the SS group means at all tests except test 3 (153). This subject had a range of scores in the emotional response domain from 137-157 with the highest scores being recorded at test 4. This subject had higher emotional response scores than the SS group mean at tests 2 (150) and 4 (157) and lower scores than the SS group mean at tests 1 (137) and 3 (149). The SSS for this subject ranged from 39-59, with the highest score being recorded at test 2. Her SSS were lower than the SS group mean at all testing times except at test 2 where her score (59) was equal to the SS group mean. This subject had TSSs which ranged from 503-543 with the highest score being recorded at test 3. The TSS for this subject were higher than the SS group mean at test 2 (527) and test 3 (543) and lower than the SS group mean at test 1 (505) and 4 (503). The high TSS at test 3 may reflect that this was the time of the highest level competition that this athlete attended. At test 4, when all of the other athletes were preparing for Nationals, this subject was finished the competitive phase of her training season. The T-scores for SSS and TSS [48, 41 (test 1); 50, 44 (test 2); 41, 47 (test 3); 41, 41 (test 4)] for this subject indicate that she had an accurate perception of her stress at all testing times.

The WOC results revealed that this athlete used planful problem solving [0.14 (test 1), 0.16 (test 2), 0.17 (test 3), 0.22 (test 4)] and positive reappraisal [0.17 (test 1),

0.21 (test 2), 0.18 (test 3), 0.18 (test 4)] as 2 of her top 3 coping strategies at all testing sessions. Other strategies used as her top 3 methods of coping were confrontive coping [0.14 (test 1), 0.19 (test 2)], accepting responsibility [0.15 (test 2), 0.13 (test 3)], distancing [0.13 (test 3)], self-controlling [0.13 (test 3)], and seeking social support [0.22 (test 1)].

The UFC concentrations [10.98 μg/24 hours (test 1), 41.80 μg/24 hours (test 2), 9.59 μg/24 hours (test 3)] for this subject were similar at test 1 and test 3 and much higher at test 2. This may reflect that test 2 was at the time of the highest level competition that this athlete attended (Western Divisionals), or it may reflect that the first and last urine samples were inadequate since the UFC concentration at these testing times were considerably lower than the expected concentrations for 24-hour extracted urine samples. The latter explanation may be more likely. This subject had very low urine volumes at all 3 testing times [680 mL (test 1), 740 mL (test 2), 370 mL (test 3)]. However despite many reassurances that no collection times were missed, the light colour and low volume of the urine at test 3 may indicate that this subject may have missed collecting some of her urine in the 24-hour period.

This subject had her first menstrual period in early 1996 at the age of 17 years and 8 months. This subject only had 2 menstrual periods during the study, the first in October of 1996 and the second in January of 1997. At the start of the study, this subject reported having a menstrual period starting July 22, 1996. This cycle was therefore used as cycle 1 so that the subsequent interval length could be calculated. The 2 interval

lengths recorded were 74 and 104 days respectively. The menstrual flow duration was consistently 4-5 days. The 2 PBAC scores reported were 23 and 65, respectively. The very young gynecologic age of this subject coupled with her relative inexperience in the sport of synchronized swimming while training with the defending National Champion team may explain her menstrual irregularity.

Subject 108 had 2 LH pulses at test 1 and 1 at test 2. This pulse frequency is lower than the SS group mean at both testing times. The mean pulse interval was considerably lower at test 1 (170.00 minutes) than at test 2 (800.00 minutes). These pulse intervals are lower than the SS group mean at test 1 and higher than the SS group mean at test 2. Mean pulse amplitude was higher at test 1 (3.95 IU/L) than at test 2 (3.66 IU/L) and higher than the SS group mean at both testing times. Mean pulse area was lower at test 1 (368.67) than at test 2 (651.63); these values were higher than the SS group means at both testing times. The mean measured level of LH for this subject was 12.89 IU/L at test 1 and 17.72 IU/L at test 2. These values were higher than the SS group mean at both testing times. The LH pulse pattern demonstrated by this subject does not coincide with the amenorrheic athlete pattern of normal pulse amplitude and decreased pulse frequency reported by Veldhuis et al. (1985) and Loucks et al. (1989). The pulse patterns demonstrated by this subject may be more similar to those exhibited in late puberty (Ferin et al., 1993).

This subject grew taller during the study which may partially account for her increase in absoluteVO_2 max. Her energy intake of calories and macronutrients is low.

This fact, coupled with her history of primary amenorrhea may indicate that her body is diverting the available energy away from reproduction in favor of more essential processes (Wade et al., 1996). The high pulse amplitude and high mean measured LH level for this subject are consistent with patterns of high around the clock LH levels which replace nocturnal increases in gonadotropin activity in the later stages of puberty (Ferin et al., 1993).

Synchronized Swimmer 109

Subject 109 was 19.4 years of age as of January 1, 1997, her gynecologic age was 6.4 years at this time making her older and more gynecologically mature than the SS group mean.

Maximum oxygen consumption results indicated that this subject had higher absolute VO_2 max results [absolute VO_2 max = 3.06 l/min (test 1), 2.78 l/min (test 2); relative VO_2 max = 47.9 mL/kg/min (test 1), 44.7 mL/kg/min (test 2)] than the SS group mean at both testing times. Her relative VO_2 max was higher than the SS group mean at test 1 and lower than the SS group mean at test 2. The decrease in her VO_2 max results from test 1 to test 2 may be a result of the final VO_2 max test being conducted after a final university exam on the day of her return from a 7-day training camp in Arizona.

Subject 109 had been training in the sport of synchronized swimming for 12 years as of May 1, 1997. She trained 11 months of the year, 6 days and an average of 19.8

hours per week. Her mean training heart rate was 107.6 bpm which is equivalent to 54% of her maximum heart rate. This athlete has competed both nationally and internationally.

This athlete was slightly taller [171.0 cm (test 1), 171.8 cm (test 2), 172.0 cm (test 3), 172.3 cm (test 4)] and weighed more [63.6 kg (test 1), 61.2 kg (test 2), 61.8 kg (test 3), 62.1 kg (test 4)] than the SS group means at all testing times. BMI for this subject [21.8 kg/m^2(test 1), 20.7 kg/m^2 (test 2), 20.9 kg/m^2 (test 3), 20.9 kg/m^2 (test 4)] was slightly higher than the SS group mean at all testing times. SOS results [68.7 mm (test1), 52.6 mm (test 2), 57.8 mm (test 3), 56.4 mm (test 4)] were higher at tests 1 and 4 than and lower at tests 2 and 3 than the SS group mean. CSTF percentile scores [35 (test 1), 65 (test 2), 55 (test 3), 60 (test 4)] were lower than the SS group mean at tests 1 and 4, higher than the SS group mean at test 2, and approximately equal to the SS group mean at test 3. BMI, SOS and CSTF percentile remained in the healthy zone for females aged 15-19 years at all testing times (CSTF Operations Manual, 1986).

Total calories consumed [2135 (test 1), 1693 (test 2), 2001 (test 3), 1814 (test 4)] were lower than the SS group mean at all testing times except at test 3 where her results were slightly higher than the SS group mean. Her protein intake as a percentage of total calories [13 % (test 1), 16 % (test 2), 9 % (test 3), 15 % (test 4)] was higher at tests 2, lower at test 3 and approximately equal to the SS group mean at tests 1 and 4. Grams of protein consumed [70 g (test 1), 70 g (test 2), 48 g (test 3), 71 g (test 4)] were lower than the SS group mean at all testing times. Her intake of carbohydrates [77 % (test 1), 66 % (test 2), 73 % (test 3), 67 % (test 4)] was higher as a percentage of the total calories

consumed than the SS group mean. Her total grams of carbohydrates consumed [430 g (test 1), 278 g (test 2), 366 g (test 3), 307 g (test 4)] were higher than the SS group mean at all testing times except test 4 where her results were equal to the SS group mean. Intake of fat as of a percentage of caloric intake and as total grams consumed [10 %, 25 g (test 1), 19 %, 36 g (test 2), 18 %, 40 g (test 3), 18 %, 39 g (test 4)] were lower than the SS group mean at all testing times.

Derogatis Stress Profile (DSP) results for this subject showed that all of her subscale scores had fairly consistent scores throughout the study and showed no consistent pattern for the small variations that did exist. This subject had personality mediators domain scores which ranged from 260-282 with the highest score recorded at test 2. All of her scores in this domain were higher than the SS groups means. This subject had environmental events domain scores which ranged from 123-127 with the highest score being recorded at test 1. This subject scored lower in this domain than the SS group means at all testing times. This subject had a range of scores in the emotional response domain from 134-157 with the highest scores being recorded at test 3. This subject had higher emotional response scores than the SS group mean at test 3 (157), lower scores than the SS group mean at tests 1 (147) and 4 (134), and a score equal to the SS group mean at test 2 (149). The SSS for this subject ranged from 60-65, with the highest score being recorded at test 2. This subject did not record SSS for tests 3 or 4. Her SSS were higher than the SS group mean at both tests that she recorded. This subject had TSS which ranged from 530-554 with the highest score being recorded at test 2. The TSS for this subject were higher than the SS group mean at tests 2 (554) and 3 (543),

lower than the SS group mean at test 1 (534), and equal to the SS group mean at test 4 (530). The T-scores for SSS and TSS [51, 45 (test 1); 53, 48 (test 2)] for this subject indicate that she had an accurate perception of her stress at both testing times that she recorded a SSS.

The WOC results revealed that this athlete used self-controlling [0.15 (test 1), 0.16 (test 2), 0.15 (test 3), 0.19 (test4)] and planful problem solving [0.26 (test 1), 0.25 (test 2), 0.29 (test 3), 0.30 (test 4)] as 2 of her top 3 coping strategies at all testing sessions. Other strategies used as her top 3 methods of coping were distancing [0.16 (test 3)], seeking social support [0.12 (test 1), 0.28 (test 4)], accepting responsibility [0.12 (test 1)], and escape avoidance [0.12 (test 1), 0.17 (test 2)].

The UFC concentrations [18.92 μg/24 hours (test 1), 26.76 μg/24 hours (test 2), 17.20 μg/24 hours (test 3)] for this subject were consistent throughout the study. This may reflect a high intensity approach to daily training, or advanced coping techniques to confront the stress of the competitive season. The UFC at test 1 and at test 3 were slightly below the expected 24-hour UFC concentration for extracted urine samples. Since the 24-hour urine volumes for this subject were relatively high (1730-1960 mL) and consistent, these low values likely do not represent inadequate urine samples. Rather, the consistency of the UFC results likely reflect a relatively constant, low level of cortisol secretion in this athlete.

The menstrual cycle characteristics of subject 109 showed consistent interval lengths throughout the study ranging from 26-32 days. Menstrual flow duration was also

consistent at 4-6 days. The PBAC scores ranged from 40-107 with the lowest score reported for cycle 5 and the highest score reported for cycle 4.

Subject 109 had 2 LH pulses both testing times. These pulse frequencies are lower than the SS group mean. The mean pulse interval was lower at test 1 (280.00 minutes) than at test 2 (400.00 minutes). These pulse intervals are lower than the SS group mean at test 1 and higher than the SS group mean at test 2. Mean pulse amplitude was higher at test 1 (6.16 IU/L) than at test 2 (4.81 IU/L) and higher than the SS group mean at both testing times. Mean pulse area was lower at test 1 (240.50) than at test 2 (357.92). Mean pulse interval values for this subject were equal to the SS group mean at test 1 and higher than the SS group mean at test 2. The mean measured level of LH for this subject was 16.99 IU/L at test 1 and 15.13 IU/L at test 2. These values were higher than the SS group mean at both testing times.

Overall, this subject was taller , heavier, and consumed fewer total calories than her team mates. Otherwise, this subject was parallel to the SS group mean on most other measures. Her relatively advanced gynecologic age, mature coping skills and experience in this sport and the training it involves may have protected this athlete from the variability in menstrual cycle patterns seen in the other case study athletes.

Summary of Case Studies

The case studies highlighted the fact that valuable individual differences may be lost when only mean data are reported. The striking finding from these case studies was

that despite similar training, aerobic fitness levels, body fat, and stress levels, one of these (108) 4 athletes was amenorrheic, 2 (101 and 103) demonstrated oligomenorrhea during the study while the other athlete (109) had regular menstrual cycles. The main difference between these athletes was their energy intake. Individual energy intakes are shown in Table 5.1. Subjects 101 and 103 ate more protein and fat than the SS group mean. This is in sharp contrast to subject 108 who consumed much less protein, fat and calories than her SS peers. Subject 109 also ate less grams of protein, less fat and more carbohydrates than her SS peers, yet this subject ate a similar number of calories to the SS group mean. These differences between the swimmers support the theory that energy intake and energy balance play an important role in achieving and maintaining menstrual function (Wade et al., 1996). There may be specific metabolic cues which are detected and affect the moment to moment pulsatility of reproductive hormones (Wade et al.). For example, subject 109 may not be consuming enough protein to synthesize the amino acids required for neurotransmitter synthesis, or enough fats to synthesize steroid hormones.

Table 5.1 Case Study Energy Intake Versus the SS Group Mean Energy Intake

ID #	Test #	Pro (g)	Pro (%)	CHO (g)	CHO (%)	Fat (g)	Fat (%)	Calories
101	1	76	13	370	63	51	21	2249
	2	94	18	263	50	66	28	2025
	3	74	15	288	59	58	26	1982
	4	103	17	322	55	75	28	2299
103	1	86	17	268	55	70	28	2009
	2	104	18	296	55	68	27	2154
	3	83	15	305	54	76	31	2152
	4	89	19	246	50	68	31	1970
108	1	40	9	372	82	20	10	1763
	2	25	10	197	78	13	11	973
	3	30	12	207	74	17	14	1060
	4	46	10	325	66	52	24	1907
109	1	70	13	430	77	25	10	2135
	2	70	16	278	66	36	19	1693
	3	48	9	366	73	40	18	2001
	4	71	15	307	67	39	18	1814
SS Group Mean	1	78.1	13.4	366.1	64.3	57.7	21.9	2242.8
	2	70.8	14.7	272.9	59.0	56.4	25.8	1854.1
	3	65.4	13.9	284.9	61.4	52.5	24.6	1892.8
	4	73.2	14.6	307.2	58.8	60.7	26.5	1989.6

Chapter Six
Summary and Conclusions

Generally, exercise leads to improved health, fitness and well-being for participants. However, some female athletes have become vulnerable to a group of medical disorders known as the female athlete triad (Nattiv et al., 1994). The female athlete triad refers to the combination of disordered eating, menstrual disorders, and decreased bone mineral density. This triad has many potential negative health consequences. One aspect of this triad, menstrual disorders was emamined in the present study. This study was unique in many ways. Previous research has not investigated all of the variables in this study at the same time with a control group. The sport and young age of the participants in this study were also unique.

Study Objectives

The primary purpose of this study was to conduct a prospective (10 month) investigation of the relationships among 24-hour urinary cortisol, energy intake, body composition, and training on the menstrual cycles of elite female synchronized swimmers. To investigate these relationships, the following parameters were investigated for 10 months in 9 elite female synchronized swimmers and 8 sedentary females:

1) 24-hour urinary cortisol levels: Urine was collected for a 24-hour period 3 times during the study and analyzed for urinary free cortisol (UFC). UFC was compared between groups and between testing times.

2) Psychological stress: Two questionnaires (the Derogatis Stress Profile and the Ways of Coping Questionnaire) were administered 4 times during the study. Subscales for types of stress and coping styles were compared between groups and between testing times.

3) Energy intake of calories, protein, carbohydrate, and fat: 3-day energy intake records were completed 4 times during the study and analyzed for mean 3-day calories, grams of protein, carbohydrate, and fat, and the percentage of the total energy intake that was comprised of protein, carbohydrate, and fat. Mean values were compared between groups and between testing times.

4) Body weight, body mass index, height, and sum of skinfolds: Height, weight, and 5 skinfold thicknesses were determined 4 times during the study. These variables were compared between testing times and between groups.

5) Training volume and intensity of elite synchronized swimmers: This parameter was measured in the synchronized swimmers only. Training volumes were determined from both athlete and coach training logs, while training intensity was determined by recording training heart rates for each athlete 6-7 times during the study. Weekly training hours were recorded for each week of the study.

6) Menstrual cycle length, menstrual flow duration, and the quantity of blood lost during each menstrual period: Menstrual cycle length and menstrual period length were determined from menstrual logs. The quantity of menstrual blood lost was estimated using pictorial blood loss (PBAC) charts completed during each menstrual period for the

duration of the study (Higham, O'Brien, & Shaw, 1990). These parameters were compared within groups between cycles and groups.

7) Luteal phase salivary progesterone: Saliva samples were collected daily from day 12 of one menstrual cycle to day 1 of the subsequent cycle during 3 menstrual cycles. Saliva was analyzed for progesterone content.

8) Early follicular phase serum luteinizing hormone pulsatility: Blood samples were collected every 10 minutes for an 8 hour period during the early follicular phase of 2 menstrual cycles during the study. The blood was analyzed for serum luteinizing hormone (LH). LH pulse frequency, amplitude, area, and interval between pulses were compared between testing times and groups.

Study Findings

Main effects of group were demonstrated for height, body mass index (BMI), absolute and relative maximum oxygen consumption (VO_2 max), and 2 DSP subscales (attitude posture and health posture). The Synchronized swimmers (SS) were significantly taller and leaner than the control group (CG) at all testing sessions. The SS also had significantly higher VO_2 max results than the CG. The SS had higher attitude posture scores than the CG while the CG had higher health posture scores than the SS. Main effects of time were found for weight, body mass index (BMI) and carbohydrate grams consumed. However, multiple t-tests using the Bonferroni correction, a very conservative post hoc test, failed to show where these differences were. Significant interaction effects were demonstrated for the sum of 5 skinfold thicknesses (SOS) and Canadian Standardized Test of Fitness (CSTF) percentile ranking. Mean SOS decreased

in the SS from test 1 to test 2 and then SOS remained stable for the duration of the study. Repeated measure ANOVA tests failed to demonstrate significant main effects of group, time or interaction effects for the remaining variables. There were problems with the progesterone data that precluded analysis.

This lack of significant differences between the mean hormonal and menstrual cycle variables may indicate that this group of athletes may be protected from the menstrual disorders that are prevalent in other sports. However, some of the individual data examined in the case studies may indicate that there is a tendency towards menstrual disorders in some of the synchronized swimmers studied. Another possible explanation for the lack of significant differences may be the young age of the subjects in the present study. It is possible that the menstrual variability associated with maturation may have over shadowed any differences associated with synchronized swimming training.

The absence of the luteal phase progesterone data is unfortunate. The length and progesterone concentration in the luteal phase would have complemented the other findings of this study. It has been suggested that a disrupted luteal phase may actually be an endpoint of successful physiological adaptation to exercise training (Loucks, 1990). Therefore, the progesterone data may have separated the groups on the basis of training or united the groups on the basis of gynecologic immaturity.

The correlational statistics performed on these data failed to show high correlations between any of the variables in this study. However, some correlations between the variables were found: 1) SOS difference with hours per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 2) SOS average with hours

per week of training at pre-test ($r = -.565$, $p<.018$) and post-test ($r = -.492$, $p<.045$), 3) SOS at pre-test with training hours per week at pre-test ($r = -.658$, $p<.004$), 4) SOS at pre-test with training hours per week at post-test ($r = -.712$, $p<.001$), 5) SOS at post test with training hours per week at pre-test ($r = -.833$, $p<.000$), 6) SOS at post-test with training hours per week at post-test ($r = -.854$, $p<000$), 7) cortisol at pre-test with average training hours per week ($r = -.603$, $p<.010$), 8) cortisol at pre-test with the difference in pre-post training hours ($r = -.603$, $p<.010$), 9) calories at pre-test with cortisol at pre-test ($r = .484$, $p<.049$), 10) calories at post-test with cortisol at pre-test ($r = .506$, $p<.038$). Many of these correlations are difficult to explain and lead to speculation that the measures chosen to represent each independent variable in the correlation matrix may not have been appropriate. In addition, mean LH concentration was chosen as the representative measure for the dependent variable, menstrual function. Luteal phase progesterone may have been a better representative measure for menstrual function in the correlation matrix than early follicular phase LH, since luteal suppression may occur before amenorrhea (Loucks, 1990) and therefore may have greater variability in menstruating athletic populations.

The case studies showed important inter-individual data that were not apparent in the group means. Subject 101's results portray the hypothesized overview of adaptations to a typical training year for a SS. During the training year, her maximum oxygen consumption, weight, body fat, and energy intake remained relatively stable. Urinary free cortisol increased as this athlete came closer to the National competition. The interval between subsequent menstrual periods increased throughout the study and LH pulse

amplitude, area, and mean level decreased. This pattern of lengthening menstrual cycles has been demonstrated in other prospective studies (Bullen et al., 1985; Beitins et al., 1991). Subject 103 increased her maximum oxygen consumption and decreased her weight and body fat during this study. Her lowest body weight and body fat results coincided with her lowest caloric intake and her longest menstrual cycle length. Subject 108 grew taller during the study which may partially account for her increase in absoluteVO_2 max. Her energy intake of calories and macronutrients was low. This fact, coupled with her history of primary amenorrhea may indicate that her body is diverting the available energy away from reproduction in favor of more essential processes (Wade et al., 1996). The high pulse amplitude and high mean measured LH level for this subject were consitent with patterns of high around the clock LH levels which replace nocturnal increases in gonadotropin activity in the later stages of puberty (Ferin et al., 1993). Subject 109 was taller , heavier, and consumed fewer total calories than her team mates. Otherwise, this subject was parallel to the SS group mean on most other measures. Her relatively advanced gynecologic age, mature coping skills and experience in this sport and the training it involves may have protected this athlete from the variability in menstrual cycle patterns seen in the other case study athletes.

Recommendations for Future Research

The present study contributed to the information available on synchronized swimmers. Very few studies have investigated athletes in this sport which is typically a very successful sport for Canada internationally. The group findings of this study may be cautiously optimistic in the sporting world which has been plagued with many studies

describing the high prevalence of menstrual disorders in athletic women (Loucks & Horvath, 1985). These results are particularly encouraging given that synchronized swimming is an aesthetic, judged sport and that these sports have shown a high prevalence of disordered eating behaviours (Sundgot-Borgen, 1994) which may eventually lead to menstrual disorders (Nattiv & Lynch, 1994). However some individual data and the absence of a luteal phase marker of menstrual cycle integrity in this study should preclude hailing synchronized swimming as a sport where swimmers are protected from menstrual disorders.

Future prospective studies should investigate synchronized swimmers that are of similar age and gynecologic age to those investigated in the present study and groups that are older and have more mature reproductive axes. This design would clarify whether synchronized swimmers are protected from menstrual disorders or whether the group of synchronized swimmers examined in this study was just too young for any reproductive disruptions due to training to be apparent.

The connection between energy intake and menstrual function also warrants further investigation. Carefully controlled studies of menstrual cycle phase, energy intake, and hormonal markers should be continued to elucidate the nutritional requirements for the maintenance of reproductive function.

The energy drain hypothesis needs to be further explored in female athletes. This study suggests that the synchronized swimmers maintained their weight, while having similar energy intake and presumably higher energy output than the control group.

Future studies should quantify daily energy output in addition to body weight, body fat, and energy intake.

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Appendix A

University of Alberta
Department of Physical Education and Recreation
Volunteer Consent
The relationships of stress, diet, and body fat to the menstrual cycles of elite female athletes.
Investigators
Dr. D. Marshall, Dr. V. Harber, Dr. D.C. Cumming, and Jennifer Ringrose

I _____(Volunteer's name) am giving my consent to participate in this research study. In so doing, I understand fully all the following statements:

1. The information to be collected includes: demographic information, height, weight, 7 skinfold thickness measurements, 4 questionnaires (food frequency, menstrual history, stress, and stress coping), a 24-hour urine sample, salivary progesterone samples, blood samples, a daily training log (synchronized swimmer participants), heart rates (synchronized swimmer participants), a maximum oxygen uptake (V02max) test, and a 3-day dietary intake record. The total time required for my participation will be approximately 30 hours over a 10 month period. I will be asked to attend 5 testing sessions during this time.

2. I have been informed of the possible benefits of my participation in this research project and understand the possible risks and discomforts associated with my participation as described in the Study Information Sheets.

3. I agree that I am voluntarily participating in the study as it is described. I understand that I have the right to withdraw from the study at any time without prejudice. I understand that there is no financial remuneration for participating in this study.

4. I expect to have my confidentiality fully protected during the time of my participation in this project, in the future, and in any published results.

5. I understand that should I have any questions related to any part of my participation in this project, my questions will be answered fully and to my total satisfaction.

6. I hereby make available to Jennifer Ringrose and her committee all results obtained as a consequence of my participation in this project, whether these results are in individual or group form.

7. I further certify that all procedures in which I will be involved have been fully explained to me. I hereby declare that I am totally satisfied with these explanations.

_____	_____	_____
Volunteer name (print)	Volunteer signature	Date
_____	_____	_____
Witness name (print)	Witness signature	Date
_____	_____	_____
Investigator's name (print)	Investigator's signature	Date

PLEASE CONTACT JENNIFER RINGROSE (403) ###-#### OR Dr. DRU MARSHALL (403) ###-#### WITH ANY QUESTIONS ABOUT THE STUDY

Appendix B

University of Alberta
Department of Physical Education and Recreation
Volunteer Consent
The relationships of stress, diet, and body fat to the menstrual cycles of elite female athletes.
Investigators
Dr. D. Marshall, Dr. V. Harber, Dr. D.C. Cumming, and Jennifer Ringrose

I _____(Parent/Guardian's name) am giving my consent for _____ (volunteer's name) to participate in this research study. In so doing, I understand fully all the following statements:

1. The information to be collected includes: demographic information, height, weight, 7 skinfold thickness measurements, 4 questionnaires (food frequency, menstrual history, stress, and stress coping), a 24-hour urine sample, salivary progesterone samples, blood samples, a daily training log (synchronized swimmer participants), heart rates (synchronized swimmer participants), a maximum oxygen uptake (V02max) test, and a 3-day dietary intake record. The total time required for the volunteer's participation will be approximately 30 hours over a 10 month period. The volunteer will be asked to attend 5 testing sessions during this time.

2. I have been informed of the possible benefits of the volunteer's participation in this research project and I understand the possible risks and discomforts associated with the volunteer's participation as described in the Study Information Sheets.

3. I agree that the volunteer is voluntarily participating in the study as it is described. I understand that the volunteer has the right to withdraw from the study at any time without prejudice. I understand that there is no financial remuneration for the volunteer's participation in this study.

4. I expect to have the volunteer's confidentiality fully protected during the time of her participation in this project, in the future, and in any published results.

5. I understand that should I have any questions related to any part of the volunteer's participation in this project, my questions will be answered fully and to my total satisfaction.

6. I hereby make available to Jennifer Ringrose and her committee all results obtained as a consequence of the volunteer's participation in this project, whether these results are in individual or group form.

7. I further certify that all procedures in which the volunteer will be involved have been fully explained to me. I hereby declare that I am totally satisfied with these explanations.

_____	_____	_____
Volunteer name (print)	Volunteer signature	Date
_____	_____	_____
Parent/Guardian name (print)	Parent/Guardian signature	Date
_____	_____	_____
Witness name (print)	Witness signature	Date
_____	_____	_____
Investigator's name (print)	Investigator's signature	Date

PLEASE CONTACT JENNIFER RINGROSE (403) ###-#### OR Dr. DRU MARSHALL (403) ###-#### WITH ANY QUESTIONS ABOUT THE STUDY

Appendix C

7-DAY FOOD FREQUENCY QUESTIONNAIRE

Please indicate the average number of servings that you have of the following food items during a typical week:

An example is provided:

	HOW OFTEN?			
FOOD ITEM	# DAY	# WEEK	# MONTH	NEVER
Coffee/Tea (1 cup)	3			
Cheese (50 gm)		2		
Dining Out			1	
Vitamins				X

This person drinks coffee 3 times a day, eats cheese twice a week, dines out once a month and never uses a vitamin supplement.

A) Dairy Products

	HOW OFTEN?			
FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
Milk (1 cup) **Ho/ 2% / 1% / Sk (circle one)**				
Cheese (50 g)				
Yogurt (3/4 cup)				

B) Meat and Alternatives

	HOW OFTEN?			
FOOD ITEM	# /DAY	# / WEEK	# / MONTH	NEVER
Poultry (100 g)				
Fish (100 g)				
Meat (100 g)				
egg (1)				
other **(peanut butter, tofu, beans)**				

C) Grain Products

HOW OFTEN?

FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
Bread (1) or Bun/Pita (1/2) white/ whole wheat (circle one)				
Cereal (3/4 cup)				
Pasta/Rice (1/2 cup)				

D) Fruit

HOW OFTEN?

FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
1 med. serving or 1/2 cup juice				

E) Vegetables

HOW OFTEN?

FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
1/2 cup or salad - 1 cup				

F) Fats

HOW OFTEN?

FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
Marg/Butter/Mayo (1 tsp.)				
Salad oil/Dairy creamer (1 tbsp.)				

G) Other

HOW OFTEN?

FOOD ITEM	# / DAY	# / WEEK	# / MONTH	NEVER
Desserts/Sweets				
Alcohol (12 oz. Beer, 1.5 oz. Liquor, 4 oz. wine)				
Soft Drinks (11.5 oz)				
Coffee/Tea (1 cup)				
Vitamin Supplements				
Dining Out				

Please indicate any foods that you choose not to eat:

_____

_____

_____

Please indicate any food allergies:

_____

_____

Appenidix D

Menstrual Cycle Information

Date:_____

Identification number:_____

Date of Birth:_____ Age:_____

Please try to answer the questions below as accurately as you can. Please answer all of the questions.

1. At what age did you have your first menstrual period? State your age in years and months. For example, 12 years, 3 months (if months are unknown, please state years only).
   YEAR_____ MONTHS_____

2. Has your menstrual cycle been regular over the past 6 months? (i.e., about every 25-35 days?)
   YES_____ NO_____

3. When was the last time your menstruated?_____

4. What is the longest time you have gone without a period?_____

5. How many periods do you usually have in a year?_____

6. What is the interval of days between your periods? Indicate the number of days between day 1 (onset of flow) of a period and day 1 of a subsequent period._____

7. On average, how many days does your period last?_____

8. Has your menstrual cycle been regular from its onset?
YES_____ NO_____

IF NO: Indicate below which pattern best describes your menstrual cycle occurrence throughout your life since the onset of your first period.

Regular-becoming irregular_____
Irregular-becoming regular_____
Never Regular_____

Does the pattern you selected above repeat itself? For example, are you regular during some months of the year and irregular during others?
YES_____ NO_____

9. Can you identify events that appear to influence your menstrual cycle pattern? List the event and state how it alters the pattern.

EVENT	CHANGE IN PATTERN	CHANGE IN FLOW
e.g. Hard training in summer	Irregular	Lighter

10. Have you experienced a significant weight loss or gain (12-15 pounds) in the last 12 months?
YES_____ NO_____
(Specify weight lost or gained and time involved)_____

11. Have you ever used oral contraceptive pills?
YES_____ Are you currently using them?_____
When did you first start using them?_____
How long (in months or years) did you or have you been taking the pill for?_____
NO_____

12. Please list ANY medication that you are currently taking or that you may take during a typical year.

_____
_____
_____
_____
_____

Appendix E

General Volunteer Information

Name:_____
Birthdate:_____

Number of Years of Synchro Training:_____

Approximately how many hours per week have you trained in the past three years?_____

Number of years of other training (specify type and competitive calibre):_____

Which school are you currently attending?_____

What grade/year are you in at school?_____

Appendix F

September 18, 1996

Dear Athlete:

I am a part of a research team at the University of Alberta that is conducting a study to determine the relationship of stress, diet and body fat to the menstrual cycle of elite female athletes. It has been shown that the irregular or absent menstrual cycles that some elite athletes experience have serious health consequences. Decreased bone mineral density and an increased risk of stress fractures are the major negative consequences of menstrual disorders. It is therefore important to understand these menstrual irregularities in order to prevent them in the future.

You have been selected as a possible candidate for our study based on your results at the 1996 National Championships and your competitive experience. This study would require approximately 30 hours of your time over a 10-month period. During this study, you would have the opportunity to learn your VO_2 max, your body fatness, and three hormone levels. We will also assess your emotional stress level, training volume and intensity, and your dietary intake.

I have enclosed some further information about the study , a diagram of the study, and an informed consent form. Should you choose to participate in the study, I have also enclosed general information, menstrual history, and food frequency questionnaires for you to complete. If you have any questions about this study, please feel free to contact me at ###-####.

Sincerely,

Jennifer Ringrose

Appendix G

University of Alberta
Department of Physical Education and Sport Studies

Study Information
The relationships of stress, diet, and body fat to the menstrual cycles of elite female athletes.

Investigators
Dr. D. Marshall, Dr. V. Harber, Dr. D.C. Cumming, Jennifer Ringrose

---

Poor nutrition, stress, low body fatness, and intense training have been associated with disruption of the menstrual cycle. Serious consequences of menstrual cycle disruption include premature bone loss, increased risk of stress fractures, and infertility. In a study lasting 10 months, we will study the relationship between 24-hour urinary cortisol, nutrition, body composition, and training in the menstrual cycles of elite female synchronized swimmers.

Twenty (20) females between the ages of 17 and 24 will be asked to complete the following:

1) **Body Composition:** Height and weight will be measures in addition to 7 skinfold thicknesses. These measurements will be taken in October, December, February, and April. The total time required at each testing session for these measurements is 30 minutes. **Total time required-2hours.**

2) **Aerobic Fitness Level:** Maximum oxygen uptake (V02max) will be assessed during a progressive, incremental exercise test to exhaustion on a stationary cycle ergometer. The resistance on the cycle will be increased progressively every two minutes during the test. Using a mouthpiece and breathing valve, expired gases are collected and monitored continuously with an automated metabolic measurement system. Heart rate will be monitored with a Polar® Heart rate monitor. This test will require the strenuous or maximal effort of the volunteer. During and after the test it is possible that the volunteer may experience symptoms such as abnormal blood pressure, fainting, lightheadedness, muscle cramps or strain, nausea, and in very rare cases, heart rhythm disturbances or heart attack. This test will be conducted in September and in April. The test takes 1 hour. **Total time required-2hours.**

3) **Dietary Intake:** Four dietary intake records (3-day diet records) will be collected (October, December, February, April). Each record will require about half an hour to complete. One food frequency questionnaire will also be given during the first month of the study. This questionnaire will require half an hour to complete. **Total time required-2.5 hours.**

4) **Menstrual Cycle Characteristics:** A questionnaire regarding menstrual cycle characteristics (age of onset, frequency, duration, etc.) will be administered four times during the study (October, December, February, April). This questionnaire will require approximately 30 minutes to complete. Volunteers will also be asked to document their cycle characteristics (frequency and duration) throughout the study **Total time required-2 hours.**

5) **Progesterone Measurements:** Early morning saliva samples will be collected for approximately 24 days during 3 menstrual cycles. This hormone will be measured to determine whether or not ovulation has occurred. Samples may be stored in the volunteer's freezer. Each morning, the sampling will take about 1 minute. All samples will be collected at the end of the menstrual cycle. **Total time required-1.25 hours.**

6) **Luteinizing Hormone Measurements:** In October and April 1-4 days after the onset of menstruation, blood will be collected to determine luteinizing hormone pulse patterns. The volunteer will report to the collection site at 7:45 a.m. An intravenous catheter will be inserted into the arm by a trained nurse or physician. A small volume of blood (2 mL) will be collected from the catheter every 10 minutes for a total of 8 hours. The total volume of blood collected will be approximately 100 mL., an amount less than a blood donation and should present no risk or danger to the volunteer. During the sampling, the volunteer will be asked to complete the questionnaires which are a part of this study. The volunteer may also watch television, read, eat, or mobilize as necessary. There is a slight risk of bruising or infection from catheter insertion. These side effects are rare. Each testing session will require approximately 8.5 hours. **Total time requirement-17 hours.**

7) **Cortisol Hormone Measurements:** In October, December, and April, 1-4 days after the onset of menstruation, volunteers will be asked to collect their urine for a 24-hour period. This measurement will determine the volunteer's stress level. Time requirement during each 24-hour period is approximately 15 minutes. **Total time requirement-45 minutes.**

8) **Stress/Stress Coping Questionnaires:** In October, December, February, and April, the volunteers will be asked to complete two questionnaires. Each takes approximately 20 minutes to complete. **Total time requirement-1.75 hours.**

9) **Training Log:** The athlete volunteers in this study will be asked to complete a daily training log. This log should include training volumes and intensities. The total time requirement will be approximately 5 minutes per day for the duration of the study.

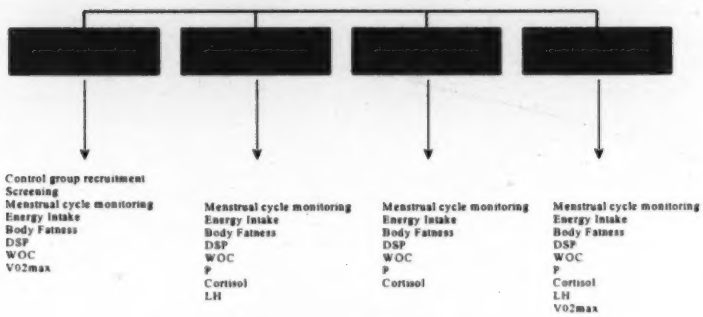
THE TOTAL TIME COMMITMENT TO COMPLETE ALL PROTOCOLS FOR THIS 10-MONTH STUDY SHOULD NOT EXCEED 30 HOURS.

The identity of each volunteer will be kept confidential. The data collected during the course of this study will be kept in a locked office at all times.

WE ENCOURAGE YOU TO ASK QUESTIONS ABOUT ANYTHING NOT UNDERSTOOD ABOUT THIS STUDY. FOR ADDITIONAL INFORMATION, PLEASE CALL DR. MARSHALL @ ###-####, DR. HARBER @ ###-####, OR JENNIFER RINGROSE @ ###-####.

Appendix H

Study Overview



Key: VO_2 max = maximum oxygen consumption, DSP = Derogatis Stress Profile, WOC = Ways of Coping Questionnaire, P = progesterone, LH = luteinizing hormone

Appendix I

INSTRUCTIONS FOR THREE DAY DIETARY RECORD
WHAT DID YOU EAT ???

You will be recording your daily intake of food and fluids for 3 consecutive days. They must be a Sunday/Monday/Tuesday or a Thursday/Friday/Saturday combination.

It is imperative that you record **EVERYTHING** that you eat and drink (water, vitamin/mineral pills as well!!!). In addition, you must be as **ACCURATE** as possible when determining the amount (volume or weight) of the food and drink you are recording. This may be difficult for those of you who have your food prepared and served by someone else, but try to be as accurate as possible. Use measuring cups/spoons and weigh scales whenever possible.

HINTS FOR RECORDING DIETARY INTAKE ACCURACY

1. Accurate Measurement: Read the weights or volumes of foods or drinks from packages. Example: milk carton, juice box, chocolate bar, potato chips. A "fistful" of
   meat = 100 gm., "fistful" veggies = 1 cup, 1 cheese single = 1 oz.

2. Method of Cooking: Indicate how your food was cooked. Example: fried, steamed, baked, broiled, etc.

3. "Extras": Don't forget the **EXTRAS.** Example: ketchup, mustard, mayonnaise, gravy, or butter.

4. Food Types: Be specific about **TYPES** of food/drink. Example: **cheddar** cheese, **2%** milk, **margarine** or **butter.** Whenever possible, identify brand names of the foods.

5. Cooked or Dry Measurement: Indicate whether the food measurement is of "cooked" or "dry". Example: chicken weight before or after cooked.

6. Specific Parts: Indicate the exact part of the food you ate or what was removed before eating. Example: chicken (white or dark, bone in or out, skin or skinless), baked potato (skin or skinless), ground beef (lean, extra lean, or regular).

7. Labels: Whenever possible, attach the nutritional information label from the container (box/can/bag). This will help identify specific brand food nutrients. If you can't remove the label, copy the information onto a piece of paper.

BEVERAGES

8. **TEA** and **COFFEE** should be included along with the cream, milk, and sugar you add.

9. Don't forget **WATER**.

10. Yes, you do have to record **BEER** and **ALCOHOL** as well.....!!

PREPARED OR RESTAURANT MEALS

11. Use **PORTION PAKS** whenever possible. Example: salad dressing, butter, jams, peanut butter, cheese. It is easier to quantify the volume of these foods... 1 portion pak = 1 tablespoon.

12. Fast Foods: Include **FAST FOOD** items by name. Example: McDonald's, Pizza Hut, Wendy's. Be sure to indicate whether you had a small, medium or large size.

13. Recipes: Record the **AMOUNT/VOLUME** of ingredients, the number of servings the entire recipe makes and how many servings you ate.

14. Restaurant Meals: When you eat at a restaurant (other than a fast food place, eg. Earl's), record the name of the meal you ate, list the different ingredients on your plate and list the quantities of each.

TAKE THE RECORDING BOOK WITH YOU AT ALL TIMES...IT'S EASIER TO RECORD WHAT YOU'RE EATING!!

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating		UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
EVENING MEAL	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Menu Item						
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home					
		Eaten Away From Your Home					
		Did Not Eat					

Day Three

	MENU ITEM Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any toppings or additives added to the menu item at the time of eating		UNIT OF MEAS. Enter the Word "cup" "ounce" "number" "teaspoon" "tablespoon"	No. of Units	DESCRIPTION OF MENU ITEM Brand	Type of Flavour	Method of Cooking
MORNING MEAL	Menu Item	eggs	number	3	Parland		scrambled
	Toppings or Additives	ketchup	tablespoon	2			
	Menu Item	sausage links	number	2	Schneider	sausage	fried
	Toppings or Additives						
	Menu Item	whole milk	cup	1	Silverwood		
	Toppings or Additives	choc mix	tablespoon	2			
	Menu Item	corn flakes	cup	2	Kellogg	corn flakes	
	Toppings or Additives	whole milk	cup	1			
		sugar	tsp.	1			
	Menu Item	banana	no.	1			
	Toppings or Additives						
	Menu Item	multi vitamin	number	1	One-A-Day		
	Toppings or Additives						
	Mark (X) One Category	Eaten at Your Home		X			
		Eaten Away From Your Home					
		Did Not Eat					

Sample Day

Appendix J

Athlete Training Log

Identification Number:_____ **Week:**_____

1) **Please complete the following chart.**
2) **Please enter volume as the number of meters, run throughs, parts, or repetitions.**
3) **Please enter duration as the number of minutes spent on each activity.**
4) **Please use the back of this sheet to document any additional information (fatigue, energy level, bad week at school, etc.) that you think may have affected your training.**

	DAY													
	Mon		Tues		Wed		Thurs		Fri		Sat		Sun	
	VOLUME	DURATION	VOLUME	DURATION	VOLUME	DURATION	VOLUME	DURATION	VOLUME	DURATION	VOLUME	DURATION	VOLUME	DURATION
Running														
Weights														
Workout														
Run Throughs														
Routine Skills														
Figure Skills														
Whole Figures														
Other (Specify)														

Appendix K

Instructions for Urine Collection

1) **Call Jennifer Ringrose (###-####) when you start menstruating so that we can arrange for your blood sampling.**

2) **Start sampling on the first full day after you start menstruating. For example, if you start menstruating on a Thursday afternoon, start collecting your urine first thing Friday morning. If you start menstruating in the night, start first thing in the morning.**

3) **Empty your bladder into the toilet first thing in the morning.**

4) **Collect all of your urine into the jug provided for a full 24 hour period including until your bladder is empty the following morning.**

5) **Try to keep the jug refrigerated (if this is not possible a cool dark place is preferred).**

6) **Once you have finished collecting, call Jennifer Ringrose and we will arrange a time to collect your jug.**

7) **Thank you for your participation.**

Appendix L

Extracted Urinary Free Cortisol Formula and Sample Calculations

The following formula is from the INCSTAR ™ GammaCoat™ Cortisol ^{125}I RIA Kit Instruction Manual.

To convert the urinary free cortisol of extracted urine samples from µg/dL to µg cortisol per 24 hours, use the following equation:

$$UFC = \frac{S \times V}{33.3\ (E-S)}$$

where:

S	=	extracted urine sample concentration in µg/dL
E	=	efficiency tube concentration for corresponding samples in µg/dL*
V	=	total urine volume in mL/24 hours
33.3	=	combined dilution and extraction efficiency factor*

* In this study, since efficiency tubes were not analyzed for each sample tube, 6.0 (the expected difference between the "E" and "S" tube concentrations in µg/dL) was used for (E-S). Therefore the formula used was $UFC = \frac{S \times V}{33.3\ (6.0)}$

Example:

S = 13.678 µg/dL
V = 740 mL/24 hours

$$UFC = \frac{(13.678)(740)}{(33.3)(6.0)} = 50.66\ \mu g \text{ cortisol / 24 hours}$$

Appendix M

Instructions for Saliva Collection

Collection Days:

Begin collection on day 12 of your menstrual cycle and continue until the onset of your next period (the first day of bleeding). This should be approximately 15-25 collection days. Saliva will be collected in October, February, and April.

Time of Collection:

Saliva should be collected in the morning, immediately upon waking and prior to eating or brushing your teeth.

Collection Procedure:

1) Rinse mouth vigorously with plain water. Wait 5 minutes.

2) Open stopper, remove cotton swab and place in mouth. Avoid handling the swab if possible.

3) Chew cotton swab for 60 seconds.

4) Gently expectorate cotton swab into the vial and close with the stopper.

5) Place vial into plastic bag (provided), place inside cardboard box. Store in freezer.

6) All vials will be collected at the end of the menstrual cycle. Please contact Jennifer Ringrose to arrange for the collection of your vials.

NOTE: All vials are numbered and it is important that they are used in the correct sequence. If for some reason a collection is missed, please make note of this, disregard that particular vial and continue the collection the next day maintaining the correct sequence. Thank you for your time.

Appendix N

Menstrual Frequency/Duration Chart

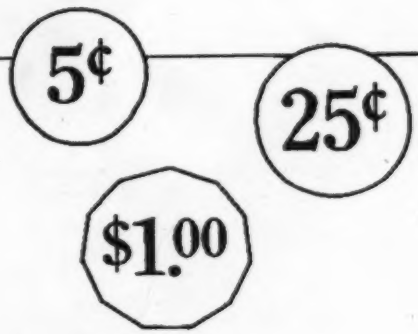
Identification Number:_____

1) Please complete the following form.
2)Please document any additional information that you think is important.

Month	Date Flow Started	Date Flow Ended	Description of flow (light, medium, heavy)	Comments (cramps, missed, etc.)
August				
September				
October				
November				
December				
January				
February				
March				
April				
May				

Appendix O

INSTRUCTIONS



Name .....

Date period started .....

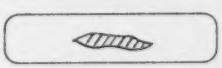
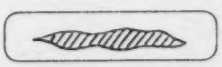
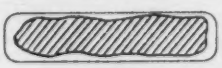
1. Enter your name and the start date (first day of your period).
2. *Before you dispose of each pad or tampon,* compare it with the pictures on the chart.
3. To record the amount of blood loss, make a mark (I) in the box opposite the picture which looks like your pad/tampon. Make a mark every time you discard a pad or tampon – on every day of your period.
4. When you reach four marks (IIII), make the next mark like this (𝍸).
5. If you notice any blood clots on the pad/tampon, or pass any in the toilet, write in the size and number each day. Guess the size by comparing the clots to coins (see examples).
6. If you experience any flooding, write F on the day it happens.
7. Please do not forget to return your completed chart(s) to your doctor. If you do not understand how to complete these charts, *please* do not be embarrassed to ask your doctor – it is important to complete the chart correctly.

PAD	1	2	3	4	5	6	7	8
CLOTS								
FLOODING								

© Sterling-Winthrop 1990

TAMPON	1	2	3	4	5	6	7	8
CLOTS								
FLOODING								

© Sterling-Winthrop 1990

PAD	1	2	3	4	5	6	7	8
	11	1	1	1111	11	1		
		1111	1111	111	1			
		1						
CLOTS		$1.00 x1 5¢ x2	5¢ x1					
FLOODING								

SAMPLE CHART
Please study this carefully with the instructions before completing your chart.

© Sterling-Winthrop 1990

Appendix P

DSP©

Name: _____ Age: _____ Sex: M____ F____ Date:_____

I.D. No: _____ Location: _____

Marital Status: Single_____ Married_____ Separated_____ Widowed_____ Divorced_____

Education: _____ Job Description: _____

INSTRUCTIONS

Below are a series of statements that describe the way some people feel about themselves. Please read each statement carefully and select one of the numbered descriptors below to indicate the extent to which the statement is true of you. Consider yourself as you **typically** behave or feel, and place the descriptor number in the open block to the right of the statement. If you change your mind, erase your first selection completely. If you have any questions, ask the technician.

DESCRIPTORS:

0 = Not at all true of me
1 = Slightly true of me
2 = Moderately true of me
3 = Very true of me
4 = Extremely true of me

1. I feel there is never enough time to get things done ☐
2. I rarely have feelings of being trapped or caught in life ☐
3. I feel rules were made to be broken ☐
4. I take some time out almost every day just to relax ☐
5. I laugh easily ☐
6. My job provides me many opportunities for challenging and satisfying activities ☐
7. When I am on vacation with my family I don't have as much fun as I think I should ☐
8. I get into frequent arguments ☐
9. I rarely feel tense and under pressure ☐
10. I rarely exercise ☐
11. I feel no interest in things ☐
12. I would like to be with my family more, but I can never seem to find the time ☐
13. I never worry about being a "workaholic" ☐
14. I believe that if you don't beat the other guy to the punch, he will beat you ☐
15. I never sit still for very long ☐
16. I am not very good at telling funny stories or jokes ☐
17. I get great pleasure from the people I work with ☐
18. I have a satisfying sex life ☐
19. I have no problems with control of my temper ☐
20. I am usually worried about something ☐
21. I smoke too much ☐
22. I rarely feel lonely ☐
23. When I eat, I usually take my time ☐
24. I frequently say I am going to spend less time on work, but I don't seem to be able to ☐
25. Most things I do I see as a challenge ☐
26. I am not very interested in hobbies or sports ☐
27. I seem to be more focused on the future than the present ☐
28. My full range of talents are not utilized on my job ☐
29. I have a good relationship with my wife/ husband (or unmarried partner) ☐
30. Sometimes I just feel like hitting somebody ☐
31. I rarely feel nervous or uptight ☐
32. I am in good physical shape ☐
33. I sometimes have feelings of worthlessness ☐
34. I rarely feel pressed for time ☐

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DSP®

DESCRIPTORS:

0 = Not at all true of me
1 = Slightly true of me
2 = Moderately true of me
3 = Very true of me
4 = Extremely true of me

35. The more things I achieve in life the less I seem to enjoy them ☐
36. I tend to be impatient. ☐
37. I sometimes just "tune out" of work and get involved in other things. ☐
38. Sex is an important part of life for me. ☐
39. I am frequently frustrated in my work. ☐
40. Interacting with my family and friends is a great source of enjoyment for me. ☐
41. I rarely have angry thoughts about people. ☐
42. When I know I have something unpleasant to do I worry about it for a long time. ☐
43. I don't take antacids for heartburn or gas. ☐
44. I usually have plenty of energy. ☐
45. I enjoy being under pressure and doing a good job on many projects at the same time. ☐
46. I really look forward to my vacations. ☐
47. I make a serious effort to achieve a balance between work and fun. ☐
48. It is not difficult for me to unwind after work. ☐
49. I really believe it is lonely at the top. ☐
50. Doing my job gives me a good feeling about myself. ☐
51. I have a good balance between family activities and work activities. ☐
52. I get easily annoyed or irritated. ☐
53. I frequently have the feeling that something bad is going to happen to me. ☐
54. I believe having good health is more important than anything. ☐
55. Sometimes I feel hopeless about the future. ☐
56. When I am driving the car, I almost never rush through traffic. ☐
57. Every day I must get something tangible accomplished or I don't feel good about myself. ☐
58. I feel the most important thing in life is that you achieve something with it. ☐
59. The idea of meditation or relaxation training has not had much appeal for me. ☐
60. I believe you can get a lot of help from others in getting the job done in life. ☐
61. There are significant parts of my job that are frankly dull and boring. ☐
62. I don't interact much with friends or neighbors. ☐
63. I rarely clench my fists during conversation. ☐
64. I rarely let things get me anxious or tense because I know they always get worked out somehow. ☐
65. I am very careful about my diet. ☐
66. I sometimes have thoughts of ending my life. ☐
67. When I have an appointment I rarely arrive late or at the last minute. ☐
68. Once I get started on a project, I don't like to stop until I am finished. ☐
69. I believe competition builds character and is good for you. ☐
70. I have trouble relaxing. ☐
71. I believe life is a struggle and you don't get anything for free out of it. ☐
72. When I wake up in the morning, I really look forward to going to work. ☐
73. I really enjoy going to parties and meeting people. ☐
74. If someone expresses a stupid idea, I rarely publicly disagree. ☐
75. Sometimes I feel tense and anxious for no apparent reason. ☐
76. I take tranquilizers to relax or sleep. ☐
77. I rarely blame myself unduly for things that go wrong. ☐

Please indicate what you believe your current level of stress to be by placing an "X" on the line below.

Totally Free of Stress ●——————————● Extremely Highly Stressed

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Appendix Q

MIND GARDEN
Palo Alto, California

Please provide the following information:

Name:_____ Date:_____
Month / Day / Year

Identification Number (optional):_____ Gender (Circle): **M** **F** Age:____

Marital Status (check): ☐ Single ☐ Married ☐ Widowed ☐ Separate/Divorced

TO THE COUNSELOR

Fill out your Institutional Address below:

_____
Name/ Institution:

_____
Address

Instructions

To respond to the statements in this questionnaire, you must have a specific stressful situation in mind. Take a few moments and think about the most stressful situation that you have experienced in the *past week*.

By "stressful" we mean a situation that was difficult or troubling for you, either because you felt distressed about what happened, or because you had to use considerable effort to deal with the situation. The situation may have involved your family, your job, your friends, or something else important to you. Before responding to the statements, think about the details of this stressful situation, such as where it happened, who was involved, how you acted, and why it was important to you. While you may still be involved in the situation, or it could have already happened, it should be the most stressful situation that you experienced during the week.

As you respond to each of the statements, please keep this stressful situation in mind. **Read each statement carefully and indicate, by circling 0, 1, 2 or 3 , to what extent you used it in the situation.**

Key: **0** = Does not apply or not used **1** = Used somewhat
2 = Used quite a bit **3** = Used a great deal

Please try to respond to every question.

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MIND GARDEN
Palo Alto, California

0 = Does not apply or not used 1 = Used somewhat 2 = Used quite a bit 3 = Used a great deal

1. I just concentrated on what I had to do next – the next step.	0	1	2	3
2. I tried to analyze the problem in order to understand it better.	0	1	2	3
3. I turned to work or another activity to take my mind off things.	0	1	2	3
4. I felt that time would have made a difference – the only thing was to wait.	0	1	2	3
5. I bargained or compromised to get something positive from the situation.	0	1	2	3
6. I did something that I didn't think would work, but at least I was doing something.	0	1	2	3
7. I tried to get the person responsible to change his or her mind.	0	1	2	3
8. I talked to someone to find out more about the situation.	0	1	2	3
9. I criticized or lectured myself.	0	1	2	3
10. I tried not to burn my bridges, but leave things open somewhat.	0	1	2	3
11. I hoped for a miracle.	0	1	2	3
12. I went along with fate; sometimes I just have bad luck.	0	1	2	3
13. I went on as if nothing had happened.	0	1	2	3
14. I tried to keep my feelings to myself.	0	1	2	3
15. I looked for the silver lining, so to speak; I tried to look on the bright side of things.	0	1	2	3
16. I slept more than usual.	0	1	2	3
17. I expressed anger to the person(s) who caused the problem.	0	1	2	3
18. I accepted sympathy and understanding from someone.	0	1	2	3
19. I told myself things that helped me feel better.	0	1	2	3
20. I was inspired to do something creative about the problem.	0	1	2	3
21. I tried to forget the whole thing.	0	1	2	3
22. I got professional help.	0	1	2	3

Go on to next page

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0 = Does not apply or not used 1 = Used somewhat 2 = Used quite a bit 3 = Used a great deal

23. I changed or grew as a person.	0	1	2	3
24. I waited to see what would happen before doing anything.	0	1	2	3
25. I apologized or did something to make up.	0	1	2	3
26. I made a plan of action and followed it.	0	1	2	3
27. I accepted the next best thing to what I wanted.	0	1	2	3
28. I let my feelings out somehow.	0	1	2	3
29. I realized that I had brought the problem on myself.	0	1	2	3
30. I came out of the experience better than when I went in.	0	1	2	3
31. I talked to someone who could do something concrete about the problem.	0	1	2	3
32. I tried to get away from it for a while by resting or taking a vacation.	0	1	2	3
33. I tried to make myself feel better by eating, drinking, smoking, using drugs, or medications, etc.	0	1	2	3
34. I took a big chance or did something very risky to solve the problem.	0	1	2	3
35. I tried not to act too hastily or follow my first hunch.	0	1	2	3
36. I found new faith.	0	1	2	3
37. I maintained my pride and kept a stiff upper lip.	0	1	2	3
38. I rediscovered what is important in life.	0	1	2	3
39. I changed something so things would turn out all right.	0	1	2	3
40. I generally avoided being with people.	0	1	2	3
41. I didn't let it get to me; I refused to think too much about it.	0	1	2	3
42. I asked advice from a relative or friend I respected.	0	1	2	3
43. I kept others from knowing how bad things were.	0	1	2	3
44. I made light of the situation; I refused to get too serious about it.	0	1	2	3

Go on to next page

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MIND GARDEN
Palo Alto, California

0 = Does not apply or not used 1 = Used somewhat 2 = Used quite a bit 3 = Used a great deal

45. I talked to someone about how I was feeling.	0	1	2	3
46. I stood my ground and fought for what I wanted.	0	1	2	3
47. I took it out on other people.	0	1	2	3
48. I drew on my past experiences; I was in a similar situation before.	0	1	2	3
49. I knew what had to be done, so I doubled my efforts to make things work.	0	1	2	3
50. I refused to believe that it had happened.	0	1	2	3
51. I promised myself that things would be different next time.	0	1	2	3
52. I came up with a couple of different solutions to the problem.	0	1	2	3
53. I accepted the situation, since nothing could be done.	0	1	2	3
54. I tried to keep my feeling about the problem from interfering with other things.	0	1	2	3
55. I wished that I could change what had happened or how I felt.	0	1	2	3
56. I changed something about myself.	0	1	2	3
57. I daydreamed or imagined a better time or place than the one I was in.	0	1	2	3
58. I wished that the situation would go away or somehow be over with.	0	1	2	3
59. I had fantasies or wishes about how things might turn out.	0	1	2	3
60. I prayed.	0	1	2	3
61. I prepared myself for the worst.	0	1	2	3
62. I went over in my mind what I would say or do.	0	1	2	3
63. I thought about how a person I admire would handle this situation and used that as a model.	0	1	2	3
64. I tried to see things from the other person's point of view.	0	1	2	3
65. I reminded myself how much worse things could be.	0	1	2	3
66. I jogged or exercised.	0	1	2	3

Stop Here.

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Appendix R

Table R.1 Subject Characteristics at Testing Time 1

ID #	Age (yrs)	Gym. Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	SOS (mm)	CSIF % ile
Synchro Group							
101	18.7	4.3	176.4	59.5	19.1	55.4	60
102	15.4	1.5	167.9	62.3	22.1	64.8	40
103	16.0	2.1	184.6	78.0	22.9	64.6	40
104	15.7	2.7	166.0	58.5	21.2	74.7	30
105	15.3	3.8	168.0	64.8	23.0	99.4	10
106	18.5	4.8	163.0	54.4	20.5	68.7	35
107	17.3	3.8	172.7	57.9	19.4	51.7	70
108	18.6	0.9	163.8	48.7	18.2	50.0	75
109	19.4	6.4	171.0	63.6	21.8	68.7	35
Mean	**17.2**	**3.4**	**170.4**	**60.9**	**21.0**	**66.4**	**43.9**
SE	**0.5**	**0.6**	**2.3**	**2.7**	**0.6**	**5.0**	**6.9**
Control Group							
201	21.3	7.8	165.2	56.6	20.7	93.6	10
202	17.5	5.4	168.1	83.7	29.6	114.0	5
203	18.8	6.8	171.1	65.5	22.6	84.2	20
204	18.6	5.2	159.5	56.7	22.3	78.4	25
205	16.7	3.7	167.6	74.0	26.3	114.7	5
206	16.8	2.4	171.5	57.1	19.4	85.3	20
207	16.7	2.9	154.5	54.4	22.8	76.2	25
208	16.8	5.8	154.0	60.0	25.3	106.6	5
Mean	**17.9**	**5.0**	**163.8**	**63.5**	**23.6**	**95.0**	**14.4**
SE	**0.6**	**0.7**	**2.5**	**3.7**	**1.2**	**5.0**	**3.2**

Appendix S

Table S.1 Menstrual Cycle Characteristics Throughout the Study for the Synchro Group

		ID #								
		101	102	103	104	105	106	107	108	109
Cycle 1	**PBAC**	DNM	DNM	DNM	DNM	DNM	DNM	DNM	DNM	DNM
	Length	5	5	5	7	DNR	7	5	5	4
	Interval	29	29	33	32	29	37	22	74	32
Cycle 2	**PBAC**	151	77	53	76	110	55	96	23	62
	Length	5	4	4	4	6	5	4	4	5
	Interval	28	46	29	32	28	29	28	104	26
Cycle 3	**PBAC**	123	137	85	44	76	55	221	65	61
	Length	5	5	5	6	6	6	6	5	6
	Interval	31	26	31	38	28	31	52	M	27
Cycle 4	**PBAC**	122	98	87	154	165	63	219	M	107
	Length	4	5	6	7	6	5	6	M	5
	Interval	34	23	31	32	28	32	27	M	28
Cycle 5	**PBAC**	DNR	121	85	145	95	129	193	M	40
	Length	4	4	5	6	5	5	7	M	4
	Interval	30	28	30	28	28	32	30	M	28
Cycle 6	**PBAC**	DNR	67	44	145	109	71	287	M	43
	Length	4	4	4	6	5	5	6	M	5
	Interval	35	34	34	27	28	38	28	M	28
Cycle 7	**PBAC**	103	96	50	139	62	66	219	M	49
	Length	4	4	5	6	7	6	5	M	6
	Interval	31	33	34	35	29	34	45	M	32
Cycle 8	**PBAC**	79	87	34	148	108	61	175	M	65
	Length	5	5	4	6	6	5	6	M	5
	Interval	39	41	61	DNM	28	DNM	30	M	27
Cycle 9	**PBAC**	128	40	DNR		DNM		177	M	53
	Length	5	4	4				5	M	5
	Interval	DNM	DNM	DNM				DNM	DNM	DNM

PBAC = PBAC score
Length = number of days period lasted
Interval = interval in days between day 1 of one cycle and day 1 of the subsequent cycle
DNM = did not measure (due to when the subject started the study, she may have missed the PBAC at the beginning or the interval at the end)
DNR = did not record (subject did not submit data)
M = missed (subject recorded data but did not menstruate during this time)

Appendix T

Table T.1 Menstrual Cycle Characteristics Throughout the Study for the Control Group

		ID #							
		201	202	203	204	205	206	207	208
Cycle 1	PBAC	227	82	247	DNR	DNM	DNR	DNM	33
	Length	8	4	6	4	6	DNR	7	5
	Interval	29	34	31	30	34	DNR	34	21
Cycle 2	PBAC	171	94	175	DNR	52	DNR	83	64
	Length	6	6	4	6	6	DNR	5	4
	Interval	27	40	29	21	28	DNR	34	46
Cycle 3	PBAC	106	83	222	DNR	74	DNR	103	53
	Length	5	4	4	DNR	6	DNR	5	5
	Interval	43	32	31	20	28	DNR	34	30
Cycle 4	PBAC	DNR	92	248	DNR	76	DNR	68	54
	Length	DNR	4	6	5	6	DNR	6	5
	Interval	45	45	31	29	28	DNR	42	DNM
Cycle 5	PBAC	DNR	136	215	DNR	DNR	DNR	DNR	
	Length	DNR	5	5	6	6	DNR	6	
	Interval	32	26	26	29	DNM	DNR	DNM	
Cycle 6	PBAC	DNR	83	244	DNR		DNR		
	Length	DNR	4	7	5		DNR		
	Interval	27	DNM	DNM	27		DNM		
Cycle 7	PBAC	DNM			301				
	Length				6				
	Interval				DNM				

PBAC = PBAC score
Length = number of days period lasted
Interval = interval in days between day 1 of one cycle and day 1 of the subsequent cycle
DNM = did not measure (due to when the subject started the study, she may have missed the PBAC at the beginning or the interval at the end)
DNR = did not record (subject did not submit data)
M = missed (subject recorded data but did not menstruate during this time)

Appendix U

Table U.1 Luteinizing Hormone Pulse Characteristics for each Subject at Both Testing Times

ID #	Pulse Frequency (# of peaks)		Mean Pulse Interval (minutes)		Mean Pulse Amplitude (IU/L)		Mean Pulse Area		Mean Measured Level (IU/L)	
	1	2	1	2	1	2	1	2	1	2
101	3	3	170.00	136.00	5.19	2.56	494.45	222.78	14.23	11.04
102	2	1	430.00	800.00	2.55	3.82	405.80	210.55	12.98	4.88
103	3	2	205.00	290.00	1.40	3.30	138.98	188.96	13.10	12.80
104	3	2	190.00	270.00	3.73	1.97	223.07	265.61	11.28	11.47
105	4	2	206.66	90.00	1.32	3.85	60.24	153.11	11.93	12.88
106	3	2	220.00	380.00	0.90	2.20	42.11	307.70	11.16	13.44
107	1	4	800.00	176.66	1.99	5.99	188.54	224.66	9.30	11.91
108	2	1	170.00	800.00	3.95	3.66	368.67	651.63	12.89	17.72
109	2	2	280.00	400.00	6.16	4.81	240.50	357.92	16.99	15.13
Mean	**2.6**	**2.1**	**296.85**	**371.41**	**3.02**	**3.57**	**240.26**	**286.99**	**12.65**	**12.36**
SE	**0.3**	**0.3**	**68.44**	**88.01**	**0.61**	**0.43**	**51.83**	**50.04**	**0.72**	**1.16**
201	3	2	270.00	270.00	1.41	6.03	133.41	395.97	11.21	10.68
202	4	3	163.33	220.00	1.63	4.72	91.64	355.55	14.09	13.00
203	2	2	410.00	230.00	2.65	3.40	129.64	290.46	12.27	13.85
204	2	2	280.00	480.00	2.49	1.43	86.99	178.03	12.13	12.56
205	3	1	245.00	790.00	2.90	1.93	92.02	158.81	12.18	11.60
206	1	1	800.00	600.00	5.82	2.91	466.84	99.80	9.15	11.91
207	1	5	800.00	127.50	2.06	3.77	314.16	340.77	7.95	23.40
208	2	3	100.00	220.00	3.16	0.90	98.45	42.11	10.71	11.16
Mean	**2.3**	**2.4**	**383.54**	**367.19**	**2.77**	**3.14**	**176.64**	**232.69**	**11.21**	**13.52**
SE	**0.4**	**0.5**	**96.29**	**81.80**	**0.49**	**0.61**	**49.23**	**46.14**	**0.69**	**1.46**

1 = testing time #1
2 = testing time #2

Appendix V

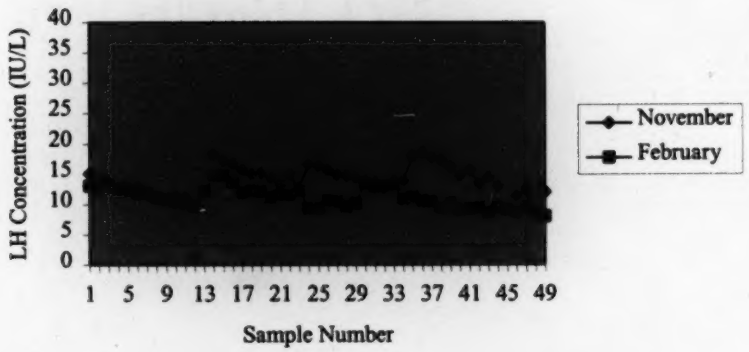

Figure V.1 Subject 101 (SS) Early Follicular Phase LH

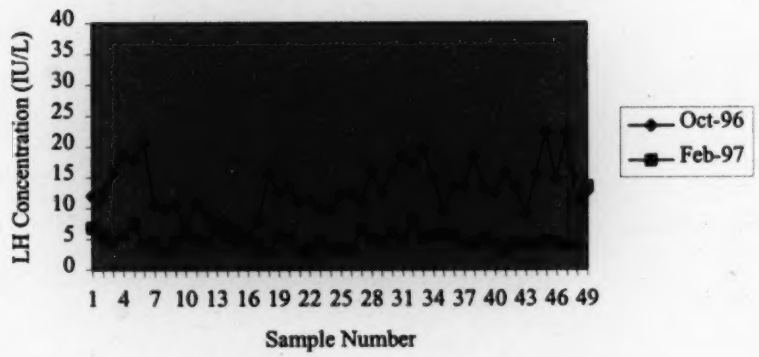

Figure V.2 Subject 102 (SS) Early Follicular Phase LH

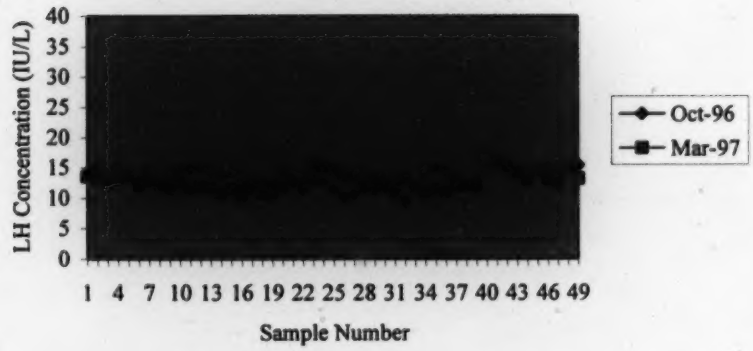

Figure V.3 Subject 103 (SS) Early Follicular Phase LH

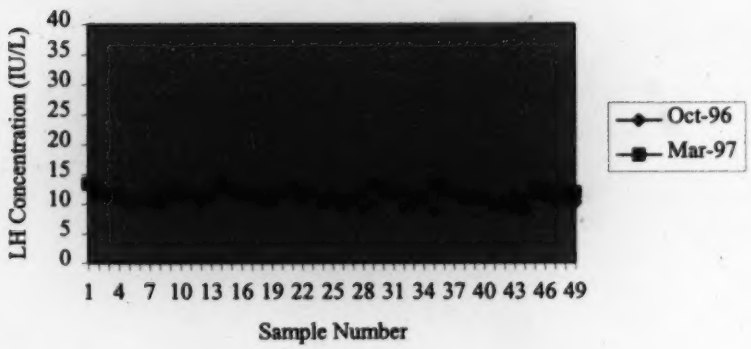

Figure V.4 Subject 104 (SS) Early Follicular Phase LH

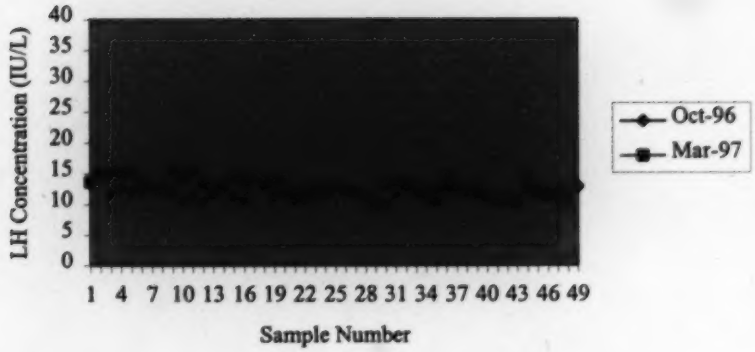

Figure V.5 Subject 105 (SS) Early Follicular Phase LH

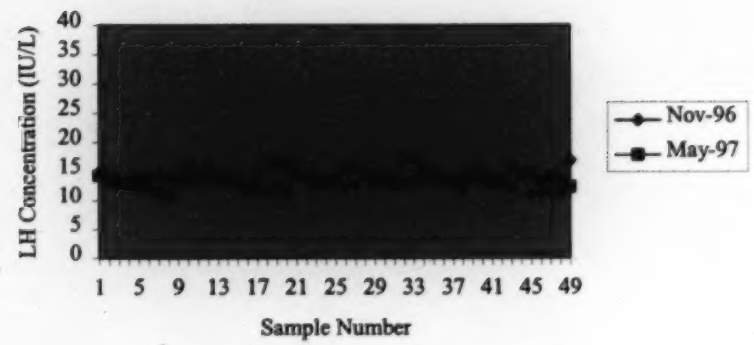

Figure V.6 Subject 106 (CG) Early Follicular Phase LH

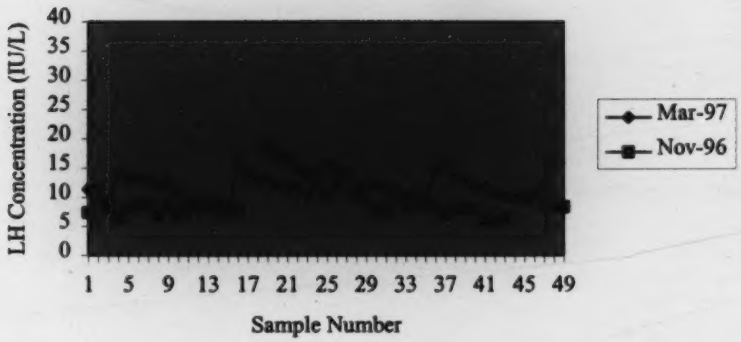

Figure V.7 Subject 107 (SS) Early Follicular Phase LH

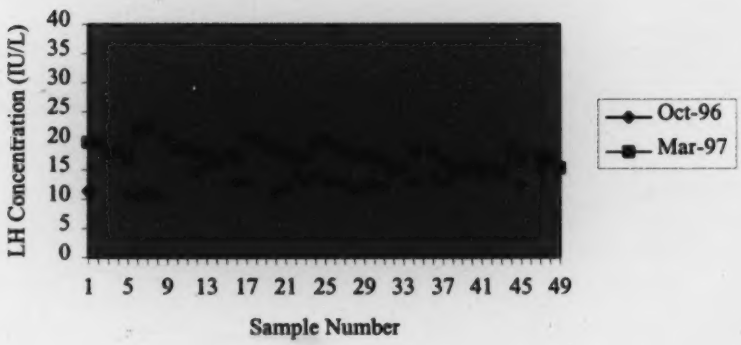

Figure V.8 Subject 108 (SS) Early Follicular Phase (October)/Arbitrary Day (March) LH

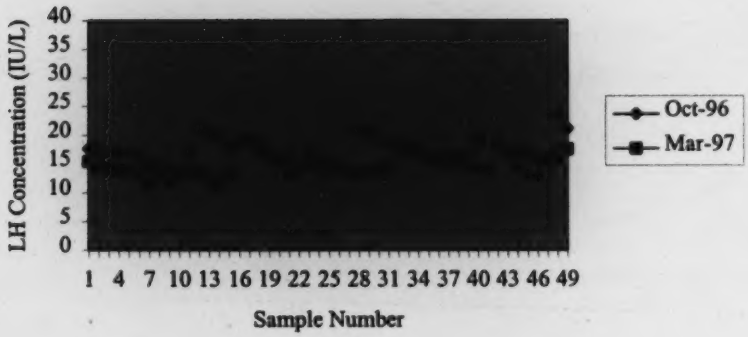

Figure V.9 Subject 109 (SS) Early Follicular Phase LH

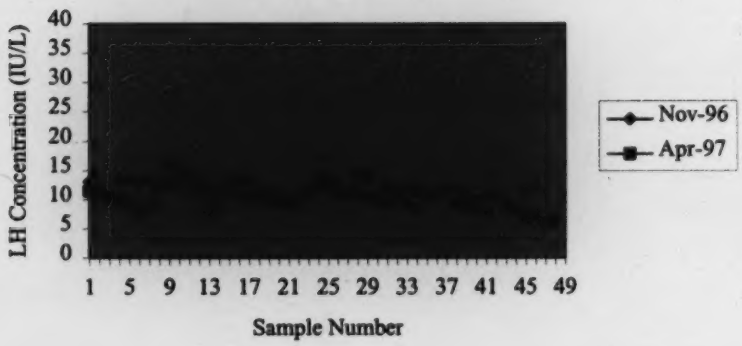

Figure V.10 Subject 201 (CG) Early Follicular Phase LH

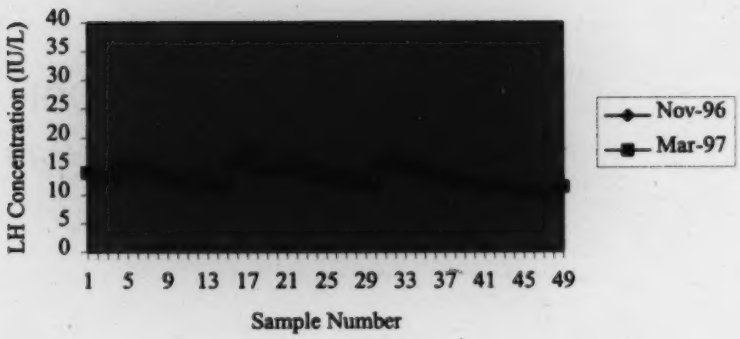

Figure V.11 Subject 202 (CG) Early Follicular Phase LH

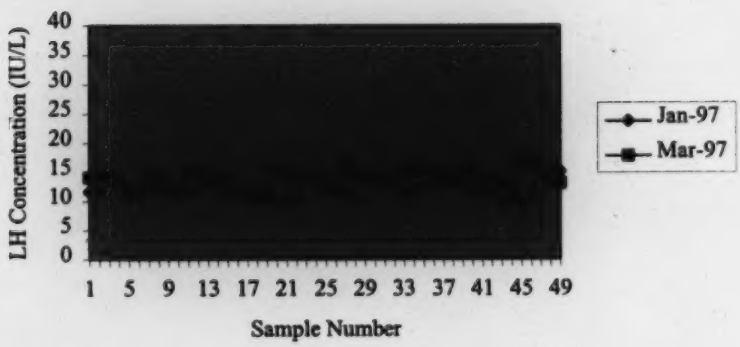

Figure V.12 Subject 203 (CG) Early Follicular Phase LH

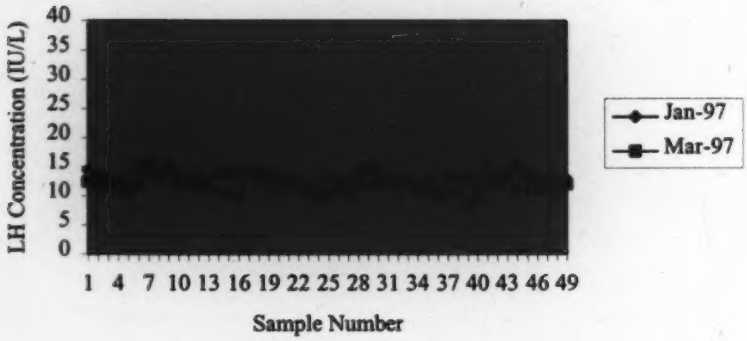

Figure V.13 Subject 204 (CG) Early Follicular Phase LH

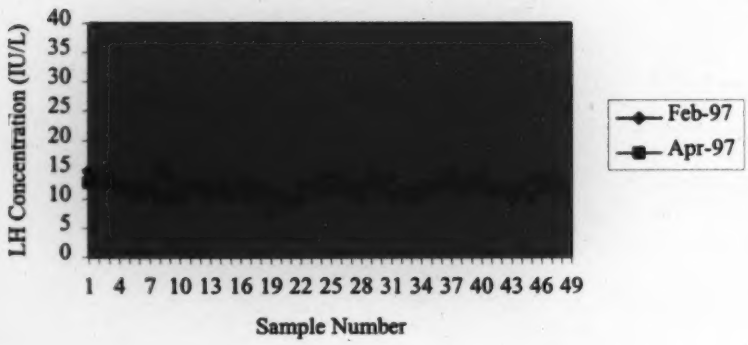

Figure V.14 Subject 205 (CG) Early Follicular Phase LH

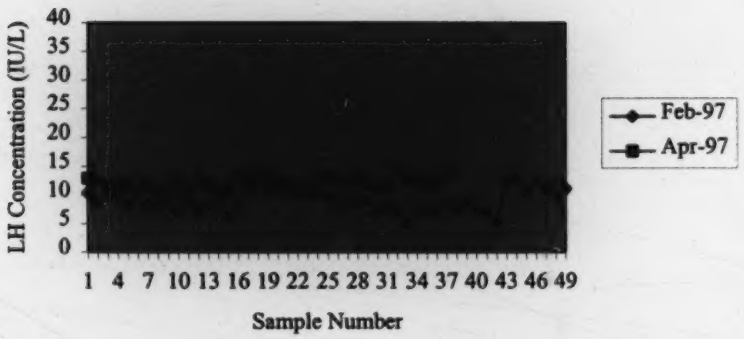

Figure V.15 Subject 206 (CG) Early Follicular Phase LH

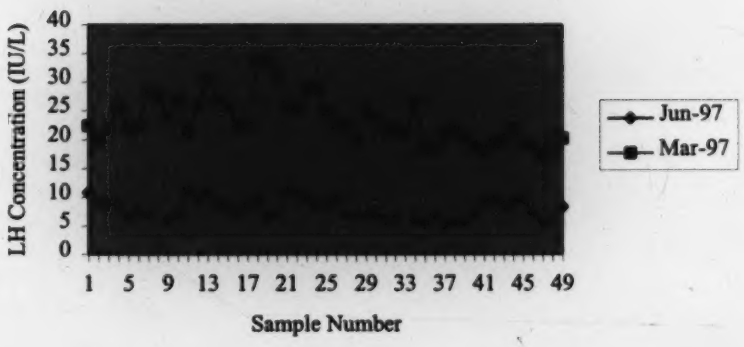

Figure V.16 Subject 207 (CG) Early Follicular Phase LH (March)/Day 41 LH (June)

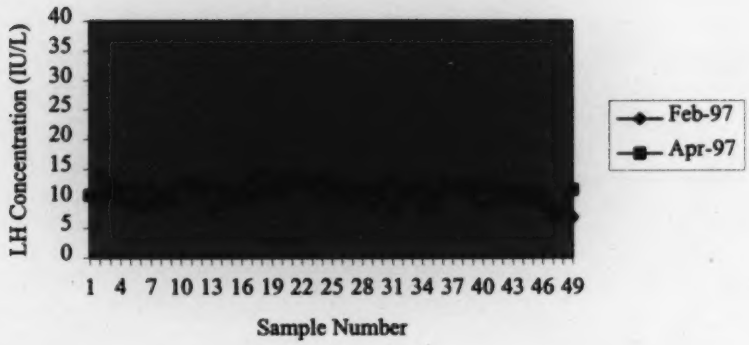

Figure V.17 Subject 208 (CG) Early Follicular Phase LH

Appendix W

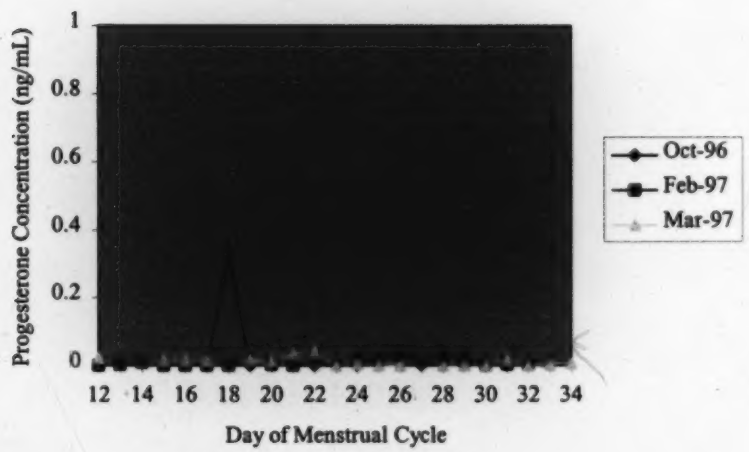

Figure W.1 Subject 101 (SS) Luteal Phase Progesterone

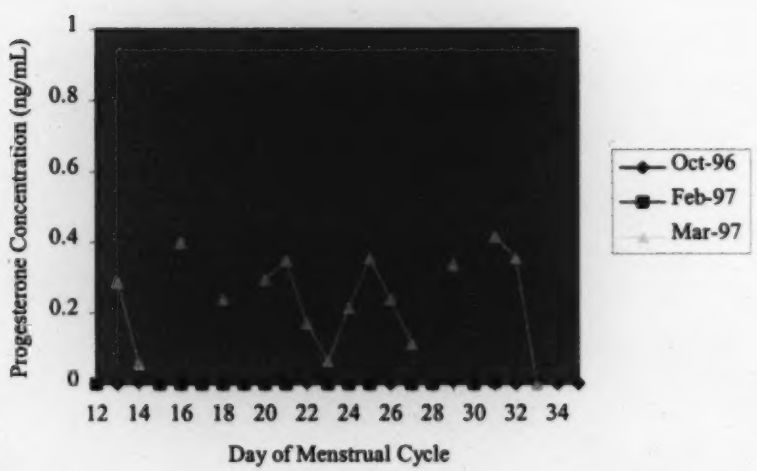

Figure W.2 Subject 102 (SS) Luteal Phase Progesterone

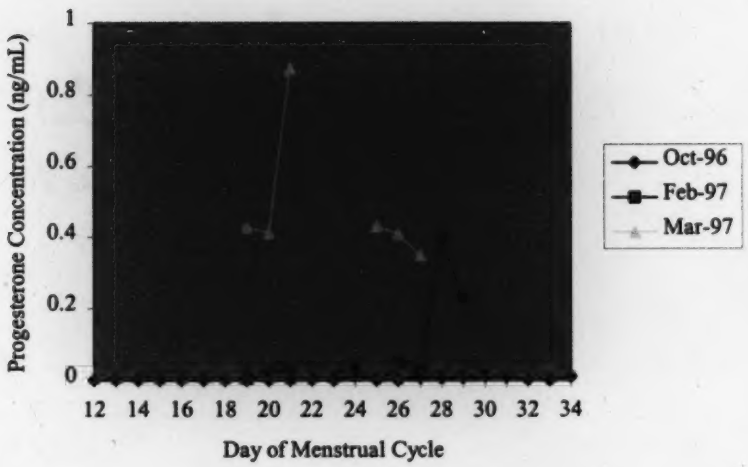

Figure W.3 Subject 103 (SS) Luteal Phase Progesterone

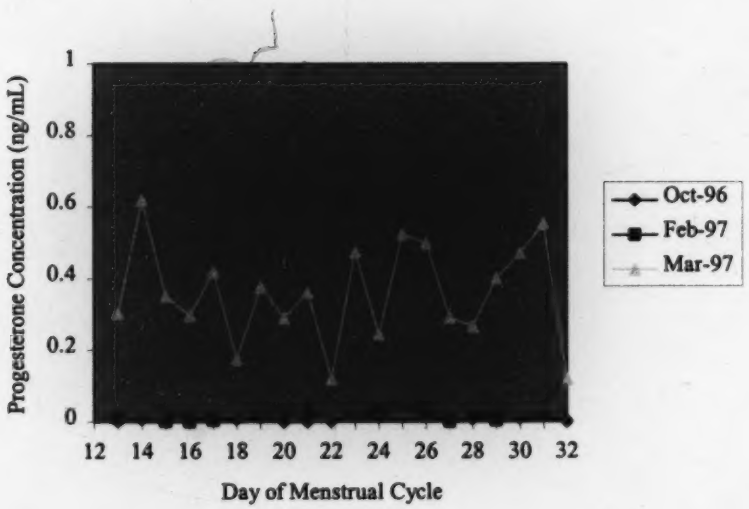

Figure W.4 Subject 104 (SS) Luteal Phase Progesterone

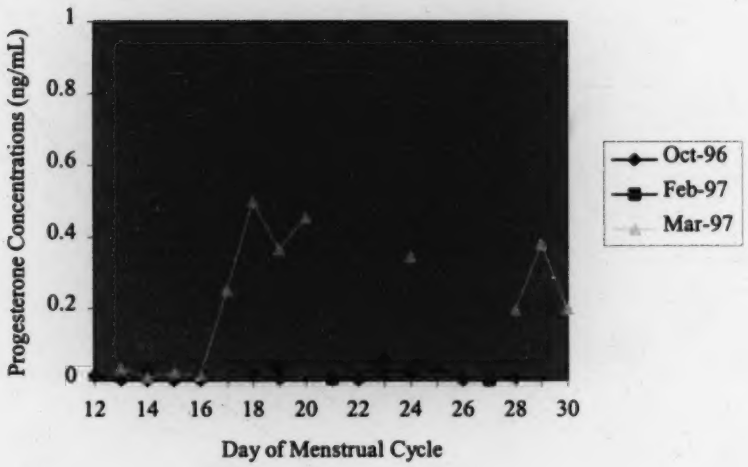

Figure W.5 Subject 105 (SS) Luteal Phase Progesterone

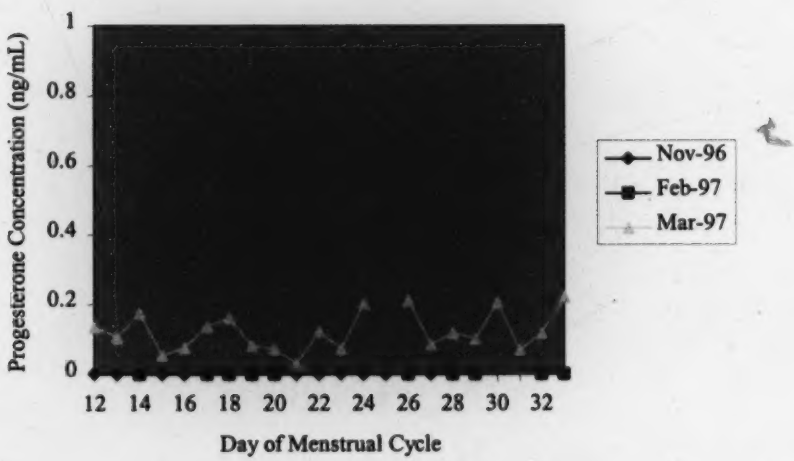

Figure W.6 Subject 106 (SS) Luteal Phase Progesterone

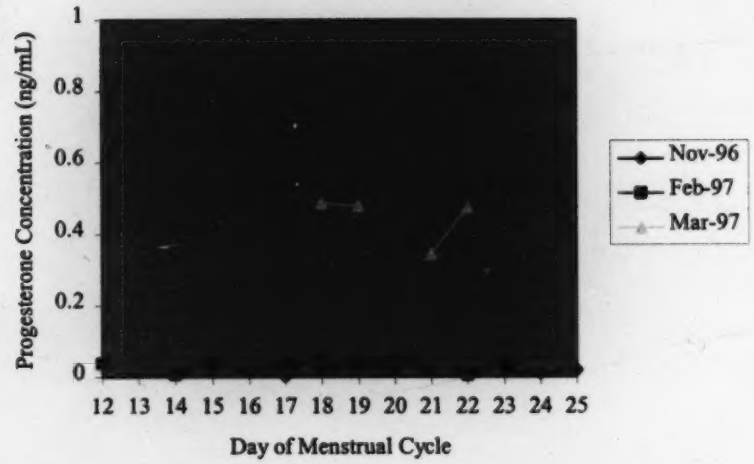

Figure W.7 Subject 107 (SS) Luteal Phase Progesterone

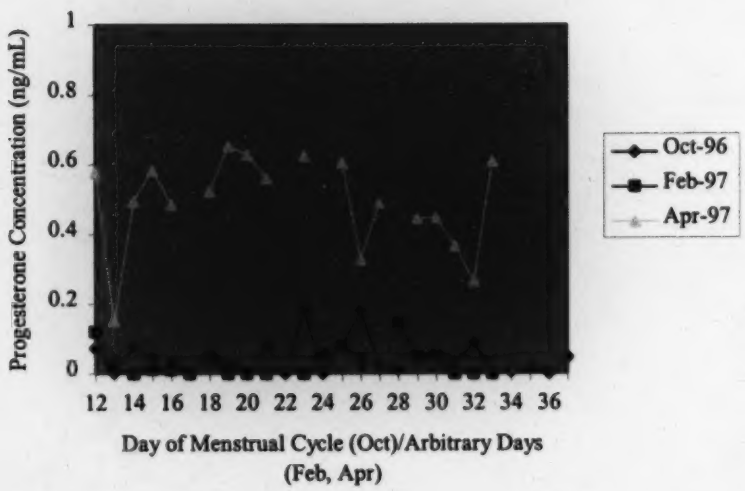

Figure W.8 Subject 108 (SS) Luteal Phase Progesterone (October)/Arbitrary Sequential days (February, April)

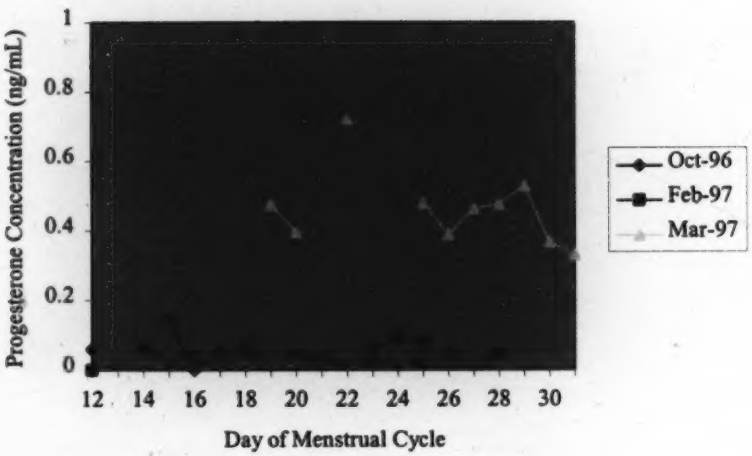

Figure W.9 Subject 109 (SS) Luteal Phase Progesterone

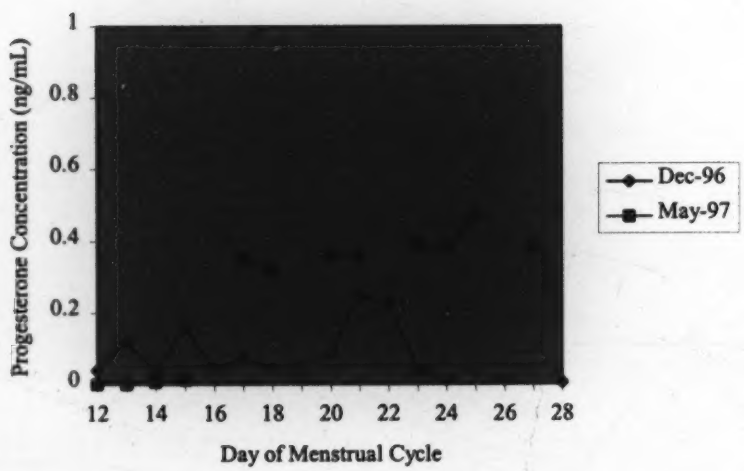

Figure W.10 Subject 201 (CG) Luteal Phase Progesterone

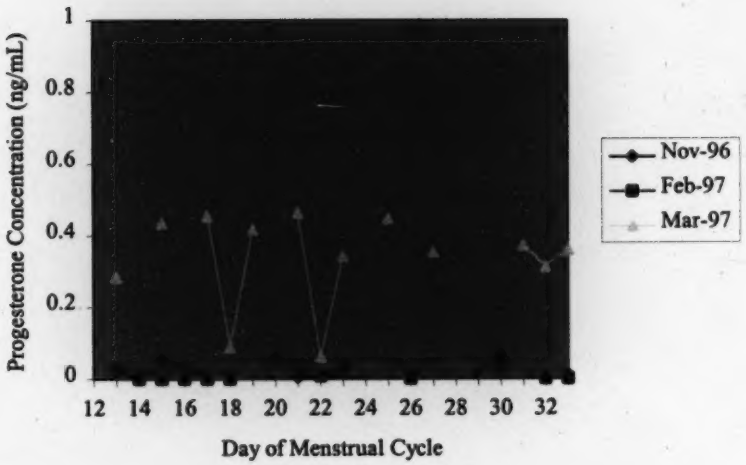

Figure W.11 Subject 202 (CG) Luteal Phase Progesterone

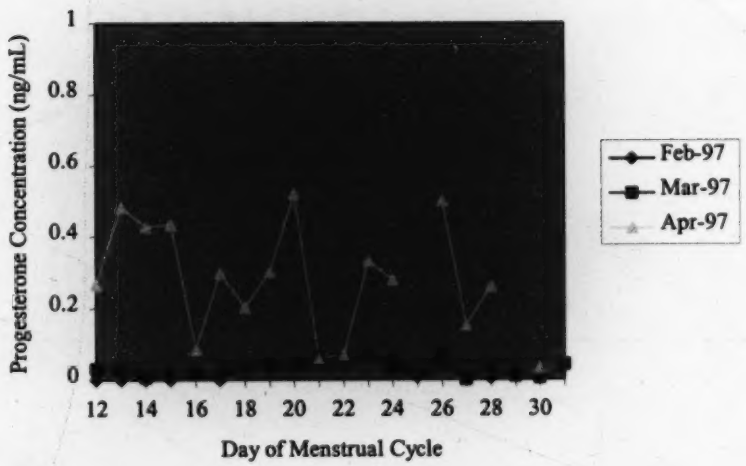

Figure W.12 Subject 203 (CG) Luteal Phase Progesterone

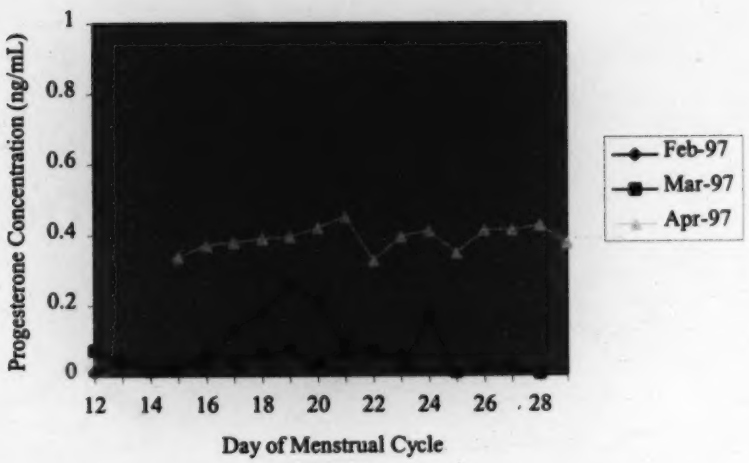

Figure W.13 Subject 204 (CG) Luteal Phase Progesterone

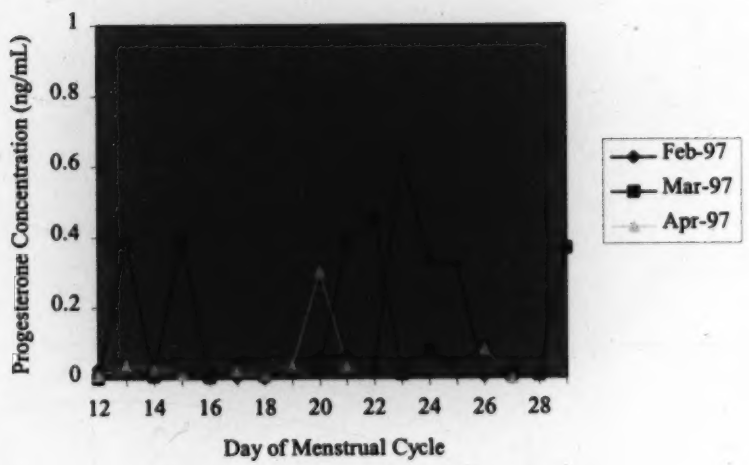

Figure W.14 Subject 205 (CG) Luteal Phase Progesterone

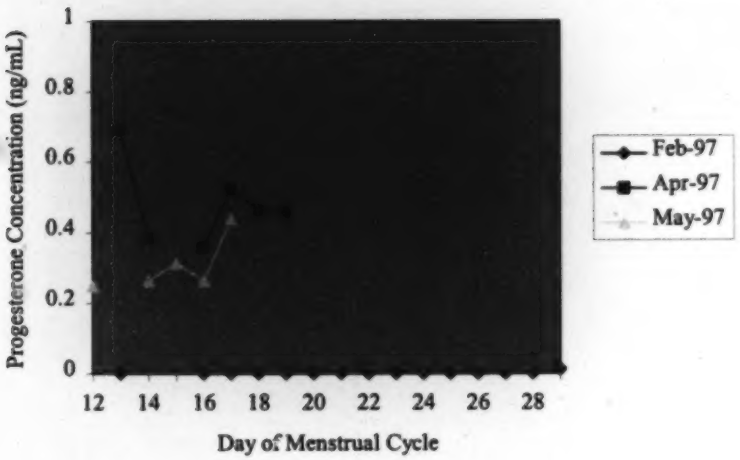

Figure W.15 Subject 206 (CG) Luteal Phase Progesterone

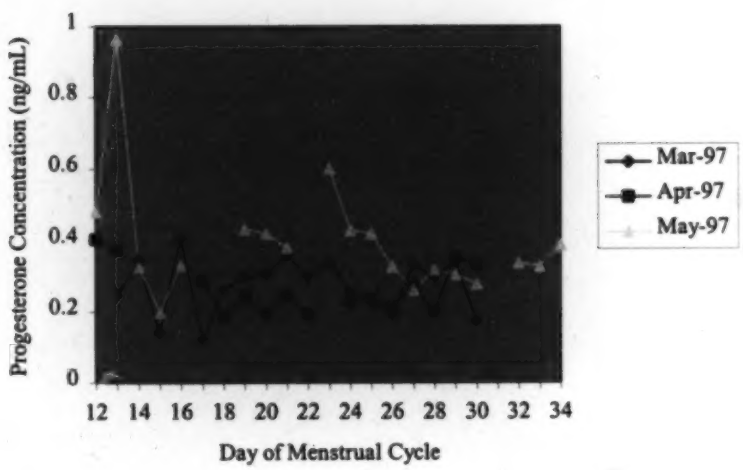

Figure W.16 Subject 207 (CG) Luteal Phase Progesterone

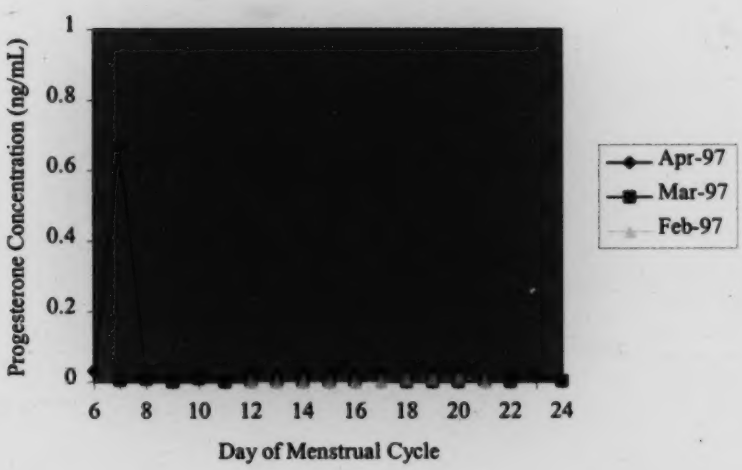

Figure W.17 Subject 208 (CG) Luteal Phase Progesterone

Appendix X

Table X.1 Pearson's Product Correlation Matrix

	LHav	SOSav	CORav	ENav	TRav	LHdif	SOSdif
LHav	1.000	.335	-.329	-.014	.044	1.000	.335
SOSav	.335	1.000	-.109	.267	.004	.335	1.000
CORav	-.329	-.109	1.000	.061	.382	-.329	-.109
ENav	-.014	.267	.061	1.000	.047	-.014	.267
TRav	.044	.004	.382	.047	1.000	.044	.004
LHdif	1.000	.335	-.329	-.014	.044	1.000	.335
SOSdif	.335	1.000	-.109	.267	.004	.335	1.000
CORdif	-.329	-.109	1.000	.061	.382	-.329	-.109
ENdif	-.014	.267	.061	1.000	0.047	-.014	.267
TRdif	.044	.004	.382	.047	1.000	.044	.004
LH1	-.627	-.268	.070	.131	.160	-.627	-.268
LH2	.894	.266	-.373	.057	.147	.894	.266
SOS1	-.042	-.013	.071	.060	-.386	-.042	-.013
SOS2	.096	.390	.022	.162	-.354	.096	.390
COR1	.174	.201	-.787	-.045	-.603	.174	.201
COR2	-.268	.121	.436	.031	-.273	-.268	.121
EN1	-.143	-.350	-.468	-.644	-.396	-.143	-.350
EN2	-.183	-.083	-.468	.466	-.400	-.183	-.083
TR1	-.307	**-.565***	.061	-.228	.177	-.307	**-.565***
TR2	-.253	**-.492***	.189	-.183	.511	-.253	**-.492***

	CORdif	ENdif	TRdif	LH1	LH2	SOS1	SOS2
LHav	-.329	-.014	.044	-.627	.894	-.042	.096
SOSav	-.109	.267	.004	-.268	.266	-.013	.390
CORav	1.000	.061	.382	.070	-.373	.071	.022
ENav	.061	1.000	.047	.131	.057	.060	.162
TRav	.382	.047	1.000	.160	.147	-.386	-.354
LHdif	-.329	-.014	.044	-.627	.894	-.042	.096
SOSdif	-.109	.267	.004	-.268	.266	-.013	.390
CORdif	1.000	.061	.382	.070	-.373	.071	.022
ENdif	.061	1.000	.047	.131	.057	.060	.162
TRdif	.382	.047	1.000	.160	.147	-.386	-.354
LH1	.070	.131	.160	1.000	-.210	-.103	-.203
LH2	-.373	.057	.147	-.210	1.000	-.112	.004
SOS1	.071	.060	-.386	-.103	-.112	1.000	.916
SOS2	.022	.162	-.354	-.203	.004	.916	1.000
COR1	-.787	-.045	-.603	-.111	.154	-.043	.041
COR2	.436	.031	-.273	-.050	-.366	.050	.095
EN1	-.468	-.644	-.396	.081	-.134	-.040	-.177
EN2	-.468	.466	-.400	.251	-.085	.027	-.009
TR1	.061	-.228	.177	.423	-.142	**-.658***	**-.833***
TR2	.189	-.183	.511	.426	-.071	**-.712***	**-.854***

	COR1	COR2	EN1	EN2	TR1	TR2
LHav	.174	-.268	-.143	-.183	-.307	-.253
SOSav	.201	.121	-.350	-.083	-.565	-.492
CORav	-.787	.436	-.468	-.468	.061	.189
ENav	-.045	.031	-.644	.466	-.228	-.183
TRav	**-.603***	-.273	-.396	-.400	.177	.511
LHdif	.174	-.268	-.143	-.183	-.307	-.253
SOSdif	.201	.121	-.350	-.083	-.565	-.492
CORdif	-.787	.436	-.468	-.468	.061	.189
ENdif	-.045	.031	-.644	.466	-.228	-.183
TRdif	**-.603***	-.273	-.396	-.400	.177	.511
LH1	-.111	-.051	.081	.251	.423	.426
LH2	.154	-.366	-.134	-.085	-.142	-.071
SOS1	-.043	.050	-.040	.027	-.658	-.712
SOS2	.041	.095	-.177	-.009	-.833	-.854
COR1	1.000	.212	**.484***	**.506***	-.085	-.289
COR2	.212	1.000	-.036	-.004	-.028	-.122
EN1	.484	-.036	1.000	.377	.265	.091
EN2	.506	-.004	.377	1.000	.030	-.116
TR1	-.085	-.028	.265	.030	1.000	.937
TR2	-.289	-.122	.091	-.116	.937	1.000

LH = LH concentrations
SOS = sum of skinfold results
COR = 24-hour urinary free cortisol
EN = energy intake
TR = training hours per week
av = the average of pre- and post test results
diff = the difference between pre-and post-test results
1 = pre-test
2 = post-test
* = significance at $p < 0.05$

Note: n=17 for each correlation in the matrix